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# Journal of the Council for Scientific and Industrial Research.

Vol. 18. NOVEMBER, 1945. No. 4.

## Drug Plant Investigations.

### 1. Progress Report.

*By C. Barnard, D.Sc.,* and H. Finnemore, B.Sc., F.R.I.C.†*

*Summary.*

A general account is given of the performance of plants producing the drugs morphine, hyoscine, atropine, hyoscyamine, ephedrine, digitalis, and santonin in plot trials, together with a description of the early phases of an investigation of the native species of *Duboisia* as sources of hyoscyamine, atropine, and hyoscine.

#### 1. Introduction.

Investigations were commenced in 1940 to determine whether certain essential medicinal drugs of plant origin could be produced successfully in Australia. This work was undertaken at the request of the Medical Equipment Control Committtee of the Army and the National Health and Medical Research Council as a war emergency precaution, and has been carried out in co-operation with the Department of Pharmacy, University of Sydney, and the Department of Physiology, University of Melbourne.

In this report, a summary of the early stages of the investigations concerned with hyoscine, atropine, hyoscyamine, morphine, ephedrine, digitalis, and santonin is presented. Accounts of later work on the opium alkaloids and the alkaloids of *Duboisia,* as well as of other drugs studies, are being prepared for separate presentation. Except where otherwise stated in the text, the chemical determinations and assays reported in this article were made by Miss Jean Kimble under the direction of Mr. H. Finnemore, and an account of the methods used in this work will be published shortly.

#### 2. Hyoscine, Atropine, and Hyoscyamine.

The principal commercial overseas sources of atropine, hyoscyamine, and hyoscine are *Atropa Belladonna, Hyoscyamus muticus,* and *Datura stramonium, Hyoscyamus niger* being used for galenical preparations only. The best sources of hyoscine are *Datura metel* and *Scopolea* spp.

Trial plots of *Atropa Belladonna* and *Hyoscyamus niger* were established, and information collated on the natural stands of *Datura* spp.

* An officer of the Division of Plant Industry.
† Reader in Pharmacy, University of Sydney.

(i) *Atropa Belladonna.*

A small plot of one square chain of *Atropa Belladonna* was established by sowing seed in the glasshouse at Canberra during July and transplanting the seedlings to the field during the latter half of October at the rate of 11,000 seedlings per acre. Flowering commenced in mid-January, and the alkaloid content (calculated as hyoscyamine) of the air-dried leaf was 0·5 per cent. at this stage and 0·43 per cent. in samples collected one month later. The plants were allowed to fruit, seed being collected, and then they were cut just above ground level. The weight of the air-dried stalk and leaf harvested was 280 lb. and the alkaloid content 0·16 per cent. It is estimated that the equivalent yield of dried leaf with an alkaloid content of 0·48 per cent. approximated 800-900 lb. per acre, which is comparable with average yields obtained overseas. Eight plants were dug during July and the root assayed 0·69 per cent. alkaloid.

During early spring of the following season, excellent growth was again made, but the plants were heavily attacked by grubs (a lepidopterous larva) and by aphis. Leaf samples collected at the end of October contained 0·65 per cent. alkaloid. Repeated sprayings with nicotine and arsenate of lead having failed adequately to control the insect pests, the plants were cut to ground level during early November. The yield of air-dried leaf was at the rate of 470 lb. per acre. Regeneration occurred rapidly, flowering took place again, and further quantities of seed were harvested. During early March, the plants were dug and a harvest of roots obtained at the rate of 1,870 lb. per acre.

(ii) *Hyoscyamus niger.*

Small plots of both an annual and biennial variety of *Hyoscyamus niger* were grown at Canberra in a manner comparable with that described for *Atropa Belladonna* above.

The biennial variety made good growth, but was attacked by the same lepidopterous larva which bored into the crown of the plants; only 66 per cent. of the plants survived the first season. The alkaloid content of the air-dried leaf (calculated as hyoscyamine) was 0·031 per cent. in samples collected during early February. During the second season, a considerable amount of "big bud" virus was evident, and aphis and grub attack continued to be severe. Leaf samples collected at flowering during early November assayed 0·066 per cent. alkaloid. No records of leaf harvests were made because of the high level of insect and virus injury.

Planting of the annual variety suffered from similar insect attack and virus infection. The alkaloid content of the leaf at flowering was only 0·022 per cent. alkaloid.

(iii) *Datura* spp.

Extensive areas of *Datura stramonium, D. tatula,* and *D. ferox* and small areas of *D. metel* and *D. Leichhardtii* occur in eastern Australia.

Samples of leaf and seed collected from different localities over three seasons showed considerable variation in alkaloid contents. Results are summarized in Table 1.

Table 1.—Alkaloid Content of Leaf and Seed Samples of *Datura* spp. from Different Localities.

| Species. | Plant Part. | Number of Samples. | Percentage Alkaloid Content* (Air-dried Samples). | |
|---|---|---|---|---|
| | | | Range. | Mean. |
| *D. stramonium* | Leaf .. | 10 | 0·18 to 0·59 | 0·43 |
| | Seed .. | 6 | 0·23 to 0·52 | 0·33 |
| *D. tatula* .. | Leaf .. | 4 | 0·39 to 0·48 | 0·43 |
| | Seed .. | 2 | 0·29 to 0·31 | 0·30 |
| *D. ferox* .. | Leaf .. | 4 | 0·23 to 0·43 | 0·33 |
| | Seed .. | 10 | 0·08 to 0·23 | 0·14 |
| *D. metel* .. | Leaf .. | 7 | 0·18 to 0·45 | 0·26 |
| | Seed .. | 2 | 0·17 to 0·47 | 0·32 |
| *D. Leichhardtii* .. | leaf, stem and fruit | 1 | .. | 0·26 |

* In *D. metel* the major alkaloid is hyoscine, and *D. Leichhardtii* also contains this alkaloid. In the other species the dominant alkaloid is hyoscyamine.

Plots established at Canberra of *Datura stramonium* from seed from nine different sources, *D. ferox* from five, and *D. metel* and *D. tatula* from three sources revealed the existence of at least three morphologically distinguishable strains of *D. stramonium* and possibly two of *D. ferox.* An insufficient number of assays was made to determine definitely whether the different strains of *D. stramonium* produced significantly different amounts of alkaloid. Assays of leaf for strain 1 were: 0·37, 0·41, 0·55, 0·58, and 0·59; for strain 2: 0·44, 0·44; and for strain 3: 0·36 and 0·39.

Further investigation of the Daturas was not pursued because the results obtained with *Duboisia,* which are described below, indicated that this genus promised to prove a better source of the mydriatic alkaloids.

(iv) *Duboisia* spp.

Sixty samples of leaf of *Duboisia myoporoides,* an indigenous tree which occurs along the eastern coast and is developed abundantly in certain locations between the Shoalhaven River in the south and Cairns in the north, were collected over a period of two years. Considerable

variation was found in the total alkaloid content of these samples, the range being from 0·62 to 3·13 per cent., with a mean of 1·86 per cent. These data are presented in Table 2.

TABLE 2.—TOTAL ALKALOID CONTENT OF AIR-DRIED LEAF OF *Duboisia myoporoides* COLLECTED IN DIFFERENT LOCALITIES.

| Location. | Alkaloid. | Location. | Alkaloid. |
|---|---|---|---|
| | % | | % |
| Tully .. .. | 1·70 | Glenreagh .. | 1·11 |
| Cairns .. .. | 0·62 | Gloucester .. | 1·06, 2·19 |
| Innisfail .. .. | 2·04 | Dorrigo .. .. | 2·05 |
| Gympie .. .. | 2·33, 2·51, 2·23, 2·03, 1·90, 2·15, 2·08, 2·34 | East Dorrigo .. | 1·58 |
| | | Coff's Harbour .. | 1·61, 2·23, 2·18 |
| | | Kempsey .. | 1·85 |
| Imbil .. .. | 1·83, 2·24, 2·24, 1·58 | Gosford .. .. | 1·81, 2·07, 2·94, 2·82, 2·52, 1·82, 1·92, 1·51, 1·72, 2·44 |
| Nambour .. | 2·99 | | |
| Yarraman .. | 3·13, 1·24, 2·86 | | |
| Goodna .. .. | 2·19 | National Park .. | 1·24, 1·20, 2·07, 1·73, 2·49, 1·92, 1·81 |
| Byron Bay .. | 2·32 | | |
| Casino .. .. | 1·77 | | |
| Springbrook .. | 1·32 | Macquarie Pass .. | 1·31, 1·16 |
| Grafton .. | 1·83, 0·91 | Kiama Berri .. | 1·22, 1·50, 0·90, 1·16 |
| Wiangaree .. | 1·40 | | |
| Brooklana .. | 1·65 | Nowra .. .. | 1·28 |

A determination of the major alkaloid was made for 22 samples; in 10 the alkaloid consisted primarily of hyoscine, and in 12 hyoscyamine was the preponderating alkaloid. The samples in which hyoscine was dominant were all collected in districts north of Gosford, and those in which hyoscyamine occurred in the Gosford area and south of Gosford. In these latter samples, hyoscine was also noted in several cases and possibly nor-hyoscyamine. Subsequent commercial extraction operations have confirmed the generalization that material collected in the north contains hyoscine, and in leaf from southern stands hyoscyamine is the major alkaloid.

Five samples of leaf of *Duboisia Leichhardtii* collected in the Yarraman-Nanango district of south-eastern Queensland contained 2·98, 2·69, 2·94, 3·02, and 3·19 per cent. alkaloid. Determination of the nature of the alkaloids present was made for the first three samples. One consisted of hyoscyamine with traces of an unidentified alkaloid, the second of hyoscyamine and hyoscine with the former preponderating, and the third of hyoscyamine and hyoscine and another alkaloid, possibly nor-hyoscyamine. Further determinations on large samples indicated the preponderance of hyoscyamine in the leaf of this species.

### 3. Morphine.

It was recognized at the outset of the investigations that the production of opium alkaloids in Australia could not be effected economically using the standard procedure of extracting from opium, because of the high labour requirement for collecting opium. A

method of direct extracting from dried material of *Papaver somniferum* along lines similar to those which had recently been developed overseas (1 and 2) seemed to offer the only possible economic means. Material required for processing in this way could be harvested by machine.

The development of a process for extracting the alkaloids direct from dried plant material was undertaken by a commercial manufacturing firm, and this aspect is not dealt with here. A colorimetric assay method which was shown by subsequent gravimetric determinations to give a reasonably accurate index of the morphine content of dried plant samples was developed and used for obtaining the results reported below. In later studies, which will be reported separately, a gravimetric method was used.

During the first season, three varieties of *Papaver somniferum* were sown in spring at Canberra (A.C.T.) and Merbein (Vic.) in 1/10th-acre plots. These varieties were:—

(*a*) a tall, white-flowered, white-seeded variety bearing a few large, sub-globular, indehiscent capsules, which has been designated "English";

(*b*) the Indian variety "Saibana" which is white-flowered, white-seeded, and bears a few small, medium sized, cylindrical and semi-indehiscent capsules; and

(*c*) a short, red-flowered Indian type, dark-seeded and producing numerous small, dehiscent capsules.

Satisfactory growth was obtained. Whole plants and capsules were harvested about fourteen days after petal fall and dried in various ways. The morphine content varied from 0·22 to 0·36 per cent. in capsules (plus seed) and from 0·11 to 0·19 per cent. in the whole plants (capsules plus straw).

Plots of varying size of these varieties were established at seven different locations by sowing in autum either in rows 3 feet apart or in 7-inch drills. The red-flowered variety was very severely damaged by frost which destroyed the crop at Canberra entirely; the Saibana variety was badly damaged, but recovered in sheltered sites and made good growth during spring; the "English" variety proved frost resistant. At Canberra, Saibana planted on the 20th of March reached full bloom during mid-October, and the "English" variety sown at the same time was in full bloom fourteen days later. The best plots of the Saibana variety yielded whole dried plant at the rate of 1 ton per acre, and of the English variety 1½ tons.

Samples of Saibana taken at approximately five-day intervals from just after petal fall (i.e., nine days after full bloom) until the plants were mature and dry *in situ* revealed but little change in the morphine content, the greatest percentage seeming to occur about three weeks

after full bloom. Samples of the English variety harvested three weeks and five weeks after full bloom showed no marked difference in morphine content. These results are set out in Table 3.

TABLE 3.—THE MORPHINE CONTENT OF SAMPLES COLLECTED AT DIFFERENT STAGES OF MATURITY.

| Variety. | Harvest in Days after Full Bloom. | Morphine in Capsules. | Morphine in Straw. | Morphine in Whole Plant. |
|---|---|---|---|---|
| | | % | % | % |
| Saibana .. .. .. | 9 | .. | .. | 0·15 |
| | 14 | 0·34 | 0·12 | 0·17 |
| | 19 | 0·31 | 0·15 | 0·20 |
| | 23 | 0·32 | 0·13 | 0·19 |
| | 29 | 0·26 | 0·14 | 0·18 |
| | 34 | 0·25 | 0·13 | 0·18 |
| English .. .. .. | 20 | 0·35 | 0·09 | .. |
| | | 0·30 | 0·11 | |
| | 33 | 0·30 | 0·08 | .. |
| | | 0·31 | 0·10 | |

Early and late bulk harvests of material grown at other locations showed no consistent difference in morphine content. There was a very small but consistently greater amount of morphine in material dried in the shade compared with material dried in the sun. For shade-dried whole plant material the morphine content averaged 0·14 per cent. and for sun-dried 0·12 per cent.; for shade-dried capsules 0·28 per cent. and sun-dried 0·25 per cent.

The average morphine contents for all samples from the seven locations at which poppies were grown are shown in Table 4.

TABLE 4.—AVERAGE PER CENT. MORPHINE IN ALL SAMPLES ASSAYED OF TWO VARIETIES.

| Location. | English Variety. | | Saibana Variety. | |
|---|---|---|---|---|
| | Morphine in Whole Plant. | Morphine in Capsules. | Morphine in Whole Plant. | Morphine in Capsules. |
| | % | % | % | % |
| Canberra, A.C.T. .. .. | 0·13(2)* | 0·31(5) | 0·18(7) | 0·30(5) |
| Merbein, Vic. .. .. | 0·16(14) | 0·29(4) | 0·14(12) | .. |
| Griffith, N.S.W. .. .. | 0·16(4) | 0·32(2) | 0·12(4) | .. |
| Margate, Tas. .. .. | .. | 0·29(5) | .. | 0·26(1) |
| Myrtleford, Vic. .. .. | 0·09(2) | 0·39(1) | 0·12(4) | .. |
| Wagga, N.S.W. .. .. | .. | .. | .. | 0·27(2) |
| Bathurst, N.S.W. .. .. | .. | 0·32(2) | .. | .. |

* The figures in brackets indicate the number of samples for which the morphine content was averaged.

The relative weight of the capsules to the whole plant increased as the capsules matured. When mature the capsules constituted from 26–30 per cent. of the weight of the whole plant (capsules plus straw) in the English variety and about 40 per cent. in the Saibana variety. In both varieties the seed constituted up to 50 per cent. of the weight of the mature capsule.

### 4. Ephedrine.

Prior to 1935, the only sources of supply of natural ephedrine were the two Chinese species *Ephedra sinica* and *Ephedra equisetina*. Seed supplies of these species could not be obtained in 1940 or subsequently. Seed was obtained, however, of three Indian species, *E. gerardiana, E. nebrodensis,* and *E. intermedia,* which had been shown to produce ephedrine (3) and had been used as sources of ephedrine since 1935, when the Japanese occupation of the Chinese-producing areas interrupted export from that country.

Plots of these three species were satisfactorily established at Canberra by sowing the seed under glass during the winter and later transplanting to the field at the rate of 5,000 or more seedlings per acre. By the spring of 1943, a total of 7,000 plants was established, the first plants being put into the field during March, 1941, and subsequent plantings being made during 1942 and 1943.

Growth and development in the field were slow in all species, especially during the first season after planting. *E. gerardiana* produced flowers and set some seed twelve months after planting, and during the second and subsequent seasons fruited very heavily. *E. intermedia* and *E. nebrodensis* did not flower or fruit until the third season from planting, and then did so only sparsely. Growth occurred mainly in two flushes, one in spring and one during autumn. The greatest bulk of vegetative growth has been made by *E. intermedia* and the least by *E. nebrodensis.* A harvest made by cutting 240 two-year-old plants of *E. gerardiana* close to ground level yielded 72 lb. of air-dried material, and 250 plants of *E. intermedia* yielded 121 lb. These yields were equivalent to approximately 2,880 lb. and 4,880 lb. per acre, respectively. Regeneration took place satisfactorily, and during the second season following cutting these plants had regained their original size.

Some variation between plants was apparent in the plots of *Ephedra gerardiana* and *E. nebrodensis,* and marked variation in form and habit was evident in the population of *E. intermedia.* Two main types of this species, a green and a blue-green form, were distinguishable.

Shoots collected during February (following a spell of dry weather) from two-year-old plants had the following total alkaloid contents* calculated as ephedrine: *E. intermedia* (blue-green) 0·58 per cent.; *E. intermedia* (green) 1·05 per cent.; *E. gerardiana* 1·35 per cent.; *E. nebrodensis* 0·98 per cent. Examinations of subsequent samples†

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* *Ephedra* assays made by Dr. F. H. Shaw, Department of Physiology, University of Melbourne, using method B.P.C. 1934, p. 410.

† These determinations made by Dr. E. Trautner of the Department of Physiology, University of Melbourne.

collected throughout the season appeared to confirm the results of Read, Feng, and Chopra (1) that the highest alkaloid content in *Ephedra* is obtained towards the end of the dry, hot season (i.e., during autumn). Variation ranged from 0·8 per cent. alkaloid in October to 1 per cent. in May in *E. intermedia* and from 0·8 per cent. to 1·5 per cent. and 1·6 per cent. in *E. gerardiana* and *E. nebrodensis*.

Plots consisting principally of *E. gerardiana*, of approximately 1 acre, have been established successfully at Armidale by the New England University College, and at Griffith under irrigation. In both locations growth and development have been comparable with that obtained at Canberra.

Although it has been reported by Ghosh and Dutt (4) that ephedrine occurs in *Sida cordifolia* in India, an examination* of material of this species growing in Australia, and also of *S. retusa, S. rhombifolia, S. intricata, S. acuta,* and *S. corrugata,* gave negative results. On the basis of the reported occurrence of ephedrine in *Taxus baccata* (5), an examination* of native species of the Taxaceae—viz., *Podocarpus alpina, Microcachrys tetragona, Dacrydium franklinii,* and *Callitris* sp.—for ephedrine was made, but negative results were obtained.

### 5. Santonin.

The usual source of santonin is the dried, unexpanded flower heads of *Artemisia cina* and other species of this genus native to Turkestan.

Six plants of *Artemisia maritima*, growing in the Plant Introduction gardens at Canberra and raised from seed obtained from Alma Ata, were multiplied by means of cuttings and a small initial plot of some hundred plants established. These plants, however, set very little viable seed and subsequent propagation was also effected by cuttings which, if taken during early spring, rooted readily. During the second and third seasons, a plot of approximately 500 plants was established both at Griffith and at Merbein under irrigation.

A sample of unexpanded flower heads collected at Canberra during the first season contained 0.53 per cent. santonin,† and two samples from Griffith during the following season 1·6 and 1·5 per cent. Samples of immature and semi-mature buds were collected at Merbein at weekly intervals from January 5 until February 6. The immature buds contained only traces of santonin, whilst the semi-mature buds contained 0·9, 0·7, 1·0, 0·7, 0·7, and 0·5 per cent.

Samples of buds collected at two stages of maturity and dried in three different ways at Griffith contained from 1·1 to 1·7 per cent. One sample collected just prior to flowering, and after some of the buds had opened, contained 2·7 per cent.

It therefore seems that the very low santonin content of the majority of the samples examined was due to premature harvesting. The B.P. standard for the dried flowers is not less than 2·0 per cent. santonin.

* These tests were made by Miss Jean Kimble, Department of Pharmacy, University of Sydney.

† Assays made by Dr. F. H. Shaw, University of Melbourne.

## 6. Digitalis.

Small plots of *Digitalis purpurea* totalling 2 square chains were grown at Canberra from seed from three sources. Seed was sown in the glasshouse during July and the seedlings transplanted to the field during September, at the rate of 7,000 per acre. Satisfactory growth was obtained and leaf collected during the first season assayed* 16, 25, and 56 per cent. above unity on the International Standard Scale for the three seed sources, respectively. The variety testing 56 per cent. above unity was Yates' Imperial foxglove, a commercial, ornamental type. After collection of seed in the second season, results being entirely satisfactory, these plots were abandoned.

A small plot of *Digitalis lanata* 1 square chain in size, established in the same manner as the plots of *D. purpurea,* developed equally well, and leaf collected during the first season of growth was equivalent to unity on the International Scale in potency. After collection of seed during the second season, this plot was also abandoned.

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* Assays made by Dr. F. H. Shaw, of the Department of Physiology, University of Melbourne; the test subject being the cat.

# Changes in the Morphine and Dry Matter Content of the Opium Poppy (*Papaver somniferum*) during the Maturation Period.

*By K. Loftus Hills, M.Agr.Sc.**

*Summary.*

Experiments were carried out at Canberra, A.C.T., to determine what changes took place in the dry matter and morphine contents of the various parts of the opium poppy plant during the period from flowering to maturity. It was found that the dry matter of husks and stems decreased as the plant matured, but that the morphine content did not vary substantially during the time when harvest of the crop would be practicable. It was also found that the upper half of the stem usually contained all the morphine, but only 20 per cent. of the dry matter of the stem.

After consideration of these and other relevant facts, it is recommended that the capsules and upper portions of the stems only should be harvested, and that generally the cutter bar of the header should be set sufficiently low to include most of the capsules, but not higher than three-quarters of the average height of the stems. The crop should be harvested as soon as the capsules are dry enough to bag directly from the header.

## I. Introduction.

An experimental programme for the production of morphine from dry poppy hay was undertaken in Australia during the war emergency (Barnard and Finnemore, 1945). Two of the more important cultivation problems concerned the stage at which the crop should be harvested and the amount of stem which might profitably be included. To answer these questions satisfactorily it is necessary to know how morphine is distributed in the plant at various stages from petal fall to capsule maturity, and to what extent the dry matter fluctuates during the same period.

Most of the earlier experimental work with the crop has involved the study of variation in the amount and quality of the dried latex, rather than of morphine in the plant tissue. Annett (1921) made a thorough investigation of factors influencing the quality and yield of Indian opium. It may be deduced from his data that the greatest amount of morphine is obtained when the capsule is lanced 16 or 17 days after petal fall. However, it should be noted that this does not necessarily mean that the morphine content of the capsule is greatest at that stage, as the questions of latex flow and residual alkaloids are also involved. Other workers have concluded that the morphine content of the opium decreases as the capsule matures.

True and Stockberger (1916) estimated the relative amounts of morphine in the several parts of the poppy plant. They found decreasing amounts in the green matter of buds, capsules, roots, leaves, and stems. More recently the morphine content of the mature straw and capsules has been determined by Bankovski and Muraveva (1942), Barnard and Finnemore (1945), Kabay (1937), Baggesgaard-Rasmussen and Salomonsen (1936) and Goris (1938). Their figures range from 0·02 to 0·15 per cent. for straw, and from 0·2 to 1·2 per cent. for husks.

In the course of a detailed study of the growth of the opium poppy in Czechoslovakia, Kuhn (1936) found that stems, leaves, and husks reached their maximum dry weight about thirteen days after petal fall.

*An officer of the Division of Plant Industry.

The experiments described in this paper were designed to determine the distribution of morphine in the principal parts of the dried opium poppy plant during the period from flowering to maturity, and to discover what changes in the dry weight of stems and husks take place during the same period.

## 2. Material.

The material for the investigation was grown at Canberra (A.C.T.) in cultivated rows, 3 feet apart. The plants were thinned within the rows, and at maturity there were generally about three plants per foot of drill row. The principal characteristics of the four varieties of *Papaver somniferum* used are shown in the following table:—

Table 1.—The Characteristics of the Four Varieties of *Papaver somniferum* used in the Experiments.

| Variety. | Maturity. | Height of Plant. | Average Number of Capsules per Plant. | Size of Capsule. | Shape of Capsule. | Dehiscence of Capsule. |
|---|---|---|---|---|---|---|
| A .. | Early mid-season | Tall .. | 1·7 | Large .. | Flat .. | — |
| B .. | Mid-season .. | Tall .. | 3·9 | Small / Medium | Conical .. | + |
| C .. | Late mid-season | Short .. | 6·0 | Small .. | Orbiculate | + |
| D .. | Late .. .. | Tall .. | 1·6 | Medium .. | Conical .. | — |

## 3. Method.

Experiments were carried out in each of the years 1942, 1943, and 1944.

In 1942, twenty uniform lengths of row of each of the varieties A and C were pegged out immediately prior to flowering. Three plants were harvested serially from each of the twenty sub-plots of each variety, at weekly intervals after petal fall. The time of petal fall varied from plant to plant, and the weekly stages represent average times only. The samples were shade-dried and the weight of straw, husk, and seed determined separately for each sample. Morphine determinations were made for the husk material from each weekly harvest.

In 1943, 250 heads of variety C were tagged when in full flower; 50 of the heads were harvested two days later, when the petals had fallen, and 50 at each of four weekly intervals thereafter. Morphine determinations were carried out on each sample. During the same year, 100 plants of uniform height of the variety D were harvested when the capsule and straw appeared to be completely dry, i.e., when the seed was mature. They were divided into stems, husks, and seed, and the stems were further divided into 6-in. lengths, the equivalent lengths of the 100 stems being bulked together to provide samples for morphine assay.

In 1944, 2,000 plants of each of the varieties A and B were tagged when the primary flower was in full bloom; 48 hours later the petals had fallen and every sixth plant was pulled up, and placed in the shade-drier. The remaining plants were lifted in a similar manner at five

weekly intervals thereafter. At the sixth sampling, both varieties had reached full maturity and were quite dry. When the remainder of the samples was dry, the capsules were removed and the seed threshed out. The length of each stem was measured and it was cut into eight equal parts. The appropriate lengths were bulked together and weighed; thus there were eight stem samples for each variety at each of six harvest times.

The leaves and roots from the plants harvested at each stage were also bulked, giving six leaf and six root samples. Morphine assays were made on these, as well as on quarter sections of the stems. The latter were obtained by bulking adjacent eights.

The morphine estimations were carried out by Miss Jean A. Kimble, under the direction of Mr. H. Finnemore, Reader in Pharmacy in the University of Sydney. The details of the method used will be published elsewhere. Assays with a different method, developed by Dr. E. M. Trautner at the School of Physiology, University of Melbourne, have shown good agreement, and it is believed that both methods give reasonably good absolute, as well as comparative, estimates of the morphine present in poppy hay. The husk samples of variety C, in 1943, were too small for the usual assay and were, therefore, done by Dr. Trautner, whose method is suitable for small amounts of raw material.

## 4. Results and Discussion.

The results of the several experiments are summarized in Tables 2, 3, 4, 5, and 6. The dry matter and morphine data will be considered separately, and then the conclusions discussed, and consideration given to their application to the problems of the optimum stage for harvesting, and the proportion of the major plant parts which should be processed.

Table 2.—The Dry Matter and Morphine Contents of the Opium Poppy Plant at Intervals from Petal Fall to Maturity at Canberra in 1942.

(*a*) *Variety "A."*

| Number of Days after Petal Fall. | Dry Matter per Plant (g.). | | | Morphine as Percentage of Dry Weight. | |
|---|---|---|---|---|---|
| | Husks. | Straw. | Seed. | Husks. | Straw. |
| 7 .. .. .. | 7·28 | 29·5 | 1·78 | 0·25 | 0·05 |
| 14 .. .. .. | 8·33 | 30·8 | 4·05 | .. | 0·05 |
| 21 .. .. .. | 7·81 | 26·2 | 5·03 | 0·26 | 0·03 |
| 28 .. .. .. | 7·72 | 31·4 | 5·28 | 0·32 | .. |
| 35 .. .. .. | 7·72 | 26·4 | 5·41 | 0·26 | 0·008 |
| Minimum difference for significance at the 5 per cent. level .. .. | .. | 4·5 | 0·94 | .. | .. |

*(b) Variety "C."*

| Number of Days after Petal Fall. | Dry Matter per Plant (g.). | | | Morphine as Percentage of Dry Weight. | |
|---|---|---|---|---|---|
| | Husks. | Straw. | Seed. | Husks. | Straw. |
| 13 .. .. .. | 3·93 | 30·6 | 3·73 | 0·62 | 0·10 |
| 19 .. .. .. | 4·04 | 25·2 | 4·47 | 0·64 | 0·07 |
| 26 .. .. .. | 3·51 | 24·6 | 3·40 | 0·62 | 0·05 |
| Minimum difference for significance at the 5 per cent. level.. .. | .. | 4·4 | 0·65 | .. | .. |

TABLE 3.—THE MORPHINE CONTENT OF HUSKS OF VARIETY C AT WEEKLY INTERVALS THROUGHOUT THE MATURATION PERIOD AT CANBERRA IN 1943.

| Number of days after petal fall .. .. .. | 2 | 9 | 18 | 25 | 32 |
|---|---|---|---|---|---|
| Morphine percentage .. | 0·65 | 0·48 | 0·47 | 0·56 | 0·52 |

TABLE 4.—THE DISTRIBUTION OF MORPHINE IN FULLY MATURE HUSKS AND STEMS OF VARIETY D AT CANBERRA IN 1943.

| Part of Plant. | Capsule. | Six-inch Segments of Stem. | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Top. | 2nd. | 3rd. | 4th. | 5th. | 6th. | 7th. | Bottom. |
| Morphine percentage | 0·46 | 0·135 | 0·060 | 0·032 | 0·006 | 0·009 | nil | nil | nil |

TABLE 5.—THE DRY MATTER AND MORPHINE CONTENTS OF THE OPIUM POPPY PLANT AT INTERVALS FROM PETAL FALL TO MATURITY AT CANBERRA IN 1944.

*(a) Variety "A."*

| Number of Days after Petal Fall. | Dry Matter per Plant (g.).* | | | Morphine as Percentage of Dry Weight. | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Husks. | Straw. | Seed. | Husks. | Top Quarter of Stem. | 2nd Quarter of Stem. | 3rd Quarter of Stem. | Bottom Quarter of Stem. | Leaf. | Root. |
| 1 .. | 1·90 | 20·3 | nil | 0·31 | 0·05 | nil | nil | nil | 0·04 | nil |
| 8 .. | 4·35 | 20·0 | 1·50 | 0·21 | .. | nil | .. | nil | 0·02 | .. |
| 15 .. | 5·28 | 19·2 | 2·25 | 0·20 | 0·11 | nil | .. | .. | 0·04 | nil |
| 22 .. | 5·09 | 18·6 | 3·02 | 0·24 | 0·09 | nil | nil | .. | nil | nil |
| 29 .. | 4·84 | 16·8 | 3·07 | 0·24 | nil | nil | .. | .. | .. | .. |
| 43 .. | 4·89 | 16·6 | 2·97 | 0·18 | nil | .. | .. | .. | nil | nil |

* With side branches removed.

(*b*) *Variety "B."*

| Number of Days after Petal Fall. | Dry Matter per Plant (g.).* | | | Morphine as Percentage of Dry Weight. | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Husks. | Straw. | Seed. | Husks. | Top Quarter of Stem. | 2nd Quarter of Stem. | 3rd Quarter of Stem. | Bottom Quarter of Stem. | Leaf. | Root. |
| 1 .. | 0·97 | 14·0 | nil | 0·58 | 0·13 | 0·09 | .. | .. | 0·10 | 0·07 |
| 8 .. | 1·52 | 14·4 | nil | 0·34 | .. | .. | .. | .. | 0·14 | .. |
| 15 .. | 1·54 | 13·8 | 0·71 | 0·42 | 0·21 | 0·09 | .. | .. | 0·19 | nil |
| 22 .. | 1·48 | 13·2 | 1·12 | 0·41 | .. | nil | .. | .. | .. | .. |
| 29 .. | 1·32 | 11·8 | 0·83 | 0·37 | .. | nil | .. | .. | nil | .. |
| 36 .. | 1·32 | 12·2 | 0·76 | .. | 0·05 | nil | .. | .. | nil | nil |

* With side branches removed.

TABLE 6.—THE EFFECT OF SPRAY IRRIGATION AT INTERVALS THROUGHOUT THE MATURATION PERIOD ON THE MORPHINE CONTENT OF THE HUSKS OF THE OPIUM POPPY.

| Number of Days after Petal Fall. | Morphine— | | Morphine Lost. |
|---|---|---|---|
| | Before Watering. | After the Application of the Equivalent of 2 in. of Rain. | |
| | % | % | % |
| 9.. .. .. .. | 0·40 | 0·33 | 17·5 |
| 16.. .. .. .. | 0·38 | 0·30 | 21·1 |
| 23.. .. .. .. | 0·39 | 0·34 | 12·8 |
| 30.. .. .. .. | 0·34 | 0·28 | 17·6 |
| 37.. .. .. .. | 0·36 | 0·29 | 19·4 |

(i) *Dry Matter.*

(*a*) *Husks.*—The changes during the maturation period in the dry weights of the husks of varieties A and C in 1942, and of A and B in 1944, are shown graphically in Fig. 1. The weights have been plotted on a logarithmic scale in order to make the changes in weight comparable in the small and in the large capsule varieties. There was a steady rise in the dry weight of the husks during the first fourteen days after petal fall, in all varieties. This rise was followed by a fall which, by full maturity, amounted to about 10 per cent. of the maximum weight. The decrease did not continue after the thirtieth day from petal fall. Kuhn (1936) records a somewhat similar decrease

in the dry weight of the husks of opium poppies grown in central Europe. The loss amounted to about 6 per cent. and began about a fortnight after petal fall. However, Kuhn observed a subsequent minor rise in weight at the end of the maturation period, which was not duplicated under Canberra conditions.

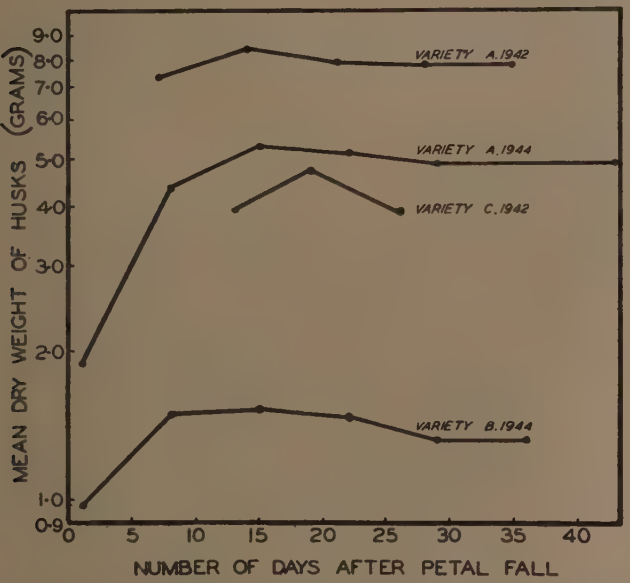


FIG. 1.—The changes in dry weight of the husks of the opium poppy during the maturation period.

Losses in dry weight by seed-containing organs during the maturation period are probably fairly common, and are the result of the translocation of reserve materials to the developing seed. Such organs are seldom of economic importance, and few investigations of their function appear to have been made. However, Bisson and Jones (1932) observed a decrease of about 40 per cent. in the dry weight of the pods of the garden pea, from the 21st to the 35th day after pollination. This represents a greater reduction in dry weight than observed in the opium poppy, but the general pattern of the weight changes during the maturation period are similar in both species.

(*b*) *Seed.*—The seed weights increased rapidly for about three weeks after petal fall. In variety A the weight then rose slightly or remained constant, but in varieties B and C, a rapid decrease in weight took place, owing to the loss of seed from the valves of the capsules, which opened about the end of the third week after petal fall.

(*c*) *Stems.*—The changes during the maturation period in the dry weights of the stems of varieties A and C in 1942, and of B and C in

1944, are represented graphically in Fig. 2. The general tendency is for the stems to lose weight steadily from the first week after petal fall to the end of the maturation period. An outstanding exception to

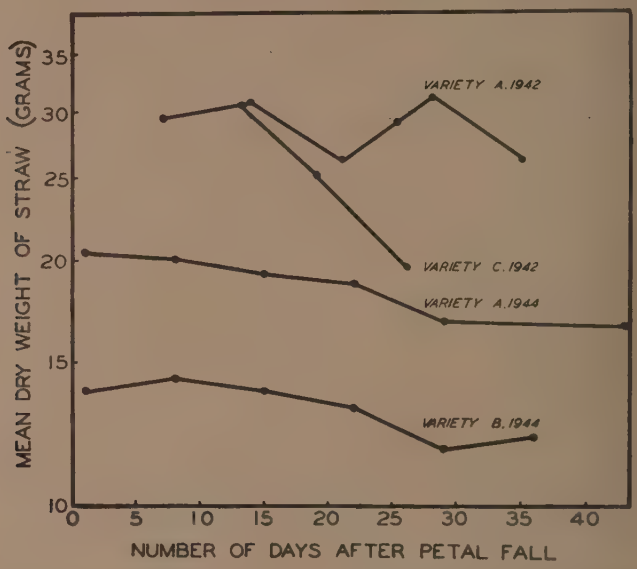


FIG. 2.—The changes in dry weight of the straw of the opium poppy during the maturation period.

the general behaviour is the substantial rise at the penultimate harvest of variety A in 1942. Statistical analysis of the figures shows that the rise is significant at the 5 per cent. level, i.e., that there is one chance in twenty of such a rise occurring purely by chance. Nevertheless it is believed to be due to chance, and not to represent a real gain in weight, because it does not conform to the remainder of the data, nor to the changes in straw weight observed in other species. The possibility of such a rise having occurred so late in the maturation period may also be dismissed on physiological grounds. In the data under review the total loss in weight amounts to about 15 per cent. of the maximum. Such decreases in the dry matter of the straw have been recorded in other species, and are the result of the transfer of reserve materials to the developing seed.

The weights of the eight fractional parts of the stem at the six stages of maturity for varieties A and B were analysed statistically to determine whether they decreased in weight in the same proportion, i.e., whether the top and bottom sections of the stem lost the same percentage of dry matter during the maturation period. This was done by comparing the interactions of the logs of the weights with the time of harvest, within and between, quarters of the stem. The interactions did not differ significantly in variety A, but did in variety B ($P = < \cdot 05$). However, inspection of the data for the latter variety

failed to reveal any general trend, and it was decided that no serious error would be introduced if the geometric means of the six harvests of each of the eight fractional parts were used to illustrate the general relationship between successive sections of stem, and their dry weights.

The geometric means of the six harvests for each fraction were then expressed as a percentage of the total dry weights of the stems, and plotted as cumulative percentages against successive stem fractions. The curves for both varieties are shown in Fig. 3. The top part of the stem contains a disproportionately small part of the dry matter, the upper half only contributing about one-fifth of the total weight.

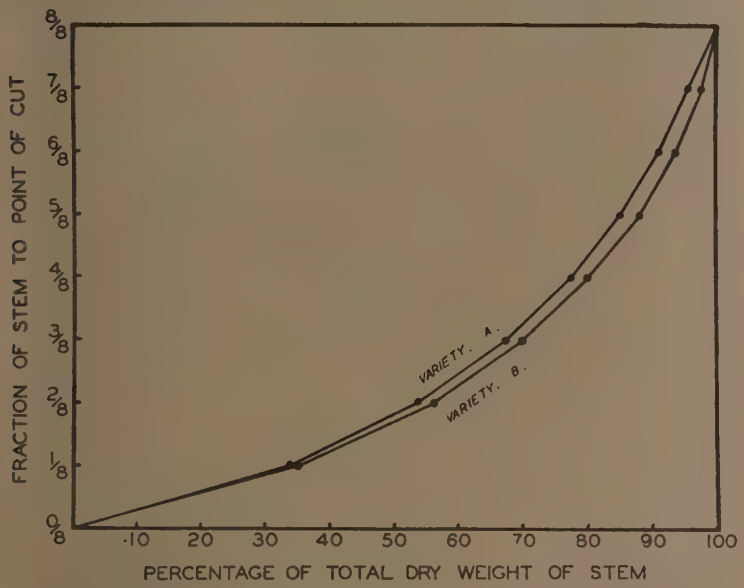


Fig. 3.—The proportion of the total dry weight of the stem of the opium poppy contributed by successive fractions from bottom to top.

(ii) *Morphine.*

(*a*) *Husks.*—The percentages of morphine in the husks of varieties A and C during the maturation period in 1942 are set out in Table 2. The morphine was fairly constant from the seventh day after petal fall onwards, although in both varieties there is evidence of a slight temporary rise about three weeks after petal fall.

The 1943 data for variety C are shown in Table 3. The first harvest was taken earlier than in 1942 and reveals a high concentration of morphine in the very young capsule. However, on the 9th day after petal fall a lower value was recorded, and this was maintained until the 18th day. An increase in morphine took place between the 18th and 25th day, and the increase was still evident, although on a reduced scale, on the 32nd day after petal fall.

The morphine content of the husks of varieties A and B grown in 1944 is shown in column 5, Table 5. The highest concentration of morphine was again found immediately after petal fall. It then fell away, but rose again during the second and third weeks. Losses of the order of 10 per cent. occurred during the fourth week in variety B, and a little later in variety A. Heavy rain fell during the maturation period and direct leaching of morphine may have taken place. Supplementary experiments, in which the standing crop was spray-irrigated at intervals throughout the maturation period, demonstrated a loss of about 15 per cent. of the morphine after the application of the equivalent of 2 inches of rain. The data are summarized in Table 6. An interesting feature of the results is the constancy of the morphine losses throughout the period. Apparently morphine is just as readily leached from the green as from the dry capsule.

The variation in the absolute weight of morphine in the husks during the maturation period is shown graphically in Fig. 4. The general tendency is for the weight of morphine per husk to rise to a

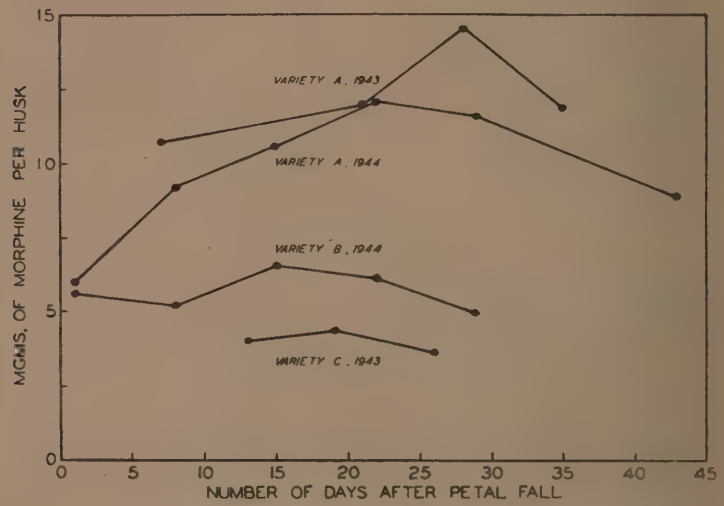


FIG. 4.—The changes in the weight of morphine per capsule during the maturation period.

maximum during the third or fourth week after petal fall; and then to decrease steadily. The time when most morphine is present seems to vary with the variety and with the conditions of growth. In three of the four cases illustrated, the peak occurred between the 15th and 22nd day after petal fall.

It would appear that under the experimental conditions described, the amount of morphine in the developing husk does not vary substantially during the third and fourth weeks after petal fall. There

is some evidence of a peak during the latter half of the maturation period, but it is somewhat irregular and of doubtful value as a guide to practical operations.

(*b*) *Stems.*—The distribution of morphine in the fully mature stems of variety D is shown in Table 4. The concentration of morphine in the top 6 inches of stem is about one-quarter of that found in the husk, and the morphine content of successively lower 6-inch lengths falls off rapidly, approximately in geometric progression. Reference to columns 6-9 of Table 5 shows a similar rapid falling off in morphine in successively lower quarters of the stems of varieties A and B. The alkaloid is apparently confined to the upper portion of the stem.

The data for the morphine content of the stem sections of varieties A and B shown in Table 5 are not complete, but there is an indication of an increase after petal fall, later followed by a decrease which in the case of variety A continued until by the sixth week after petal fall measurable amounts of morphine were no longer present.

(*c*) *Leaves and Roots.*—Morphine was not present in measurable amounts in the leaves of either variety after about the second week after petal fall, although at the beginning of the maturation period they contained about one-seventh as much morphine as did the husks (Table 5, Col. 10).

With the exception of variety B, harvested at petal fall, morphine was not found in measurable amounts in the roots of either variety. The relevant data are set out in column 11 of Table 5.

(iii) *Application of the Results.*

The changes in dry weight and percentage of morphine which take place in the opium poppy during the maturation period are not great, and certain practical considerations become the principal factors determining the optimum time at which to harvest the crop. The chief factor limiting harvest early in the maturation period is the difficulty of drying the green material. In addition, Trautner (privately communicated) has found that husks harvested early in the maturation period often contain additional pigments which under certain conditions may interfere with recovery of the alkaloid. On the other hand later harvest may result in a somewhat lower yield of morphine, and in an increased risk of direct leaching by rain. It would therefore seem desirable to harvest the crop as soon as it is dry enough to bag directly from the header. This stage was reached at Canberra in 1944 during the fourth week after petal fall.

By the middle of the maturation period, neither leaves, roots, nor the lower halves of the stems contain appreciable amounts of morphine and therefore should not be harvested. The amount of stem which can profitably be included will depend upon the variety of poppy and the conditions of growth. Within the limit indicated, larger gross amounts of morphine will be obtained by inclusion of more stem, but this will result in a more dilute raw material, which will involve greater handling and processing costs per unit of morphine recovered. This cost must be balanced against the value of the additional morphine obtained.

The rapid dilution of the raw material as greater amounts of stem are included, and the proportion of the total morphine obtained by so doing, are shown in Fig. 5. In variety A the morphine in the stem

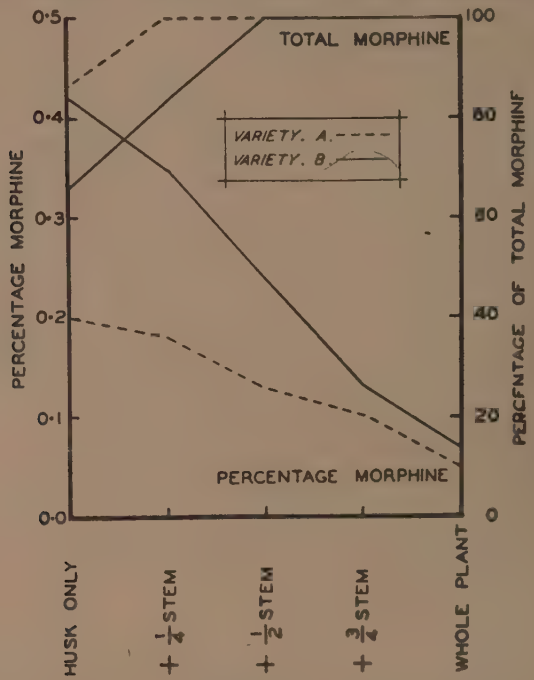


FIG. 5.—The relationship between the proportion of stem included in the harvest, the percentage of morphine in the harvested material, and the percentage of the total morphine harvested, for varieties A and B grown at Canberra in 1944.

is confined to the top quarter, and it would probably be economical to recover the additional 14 per cent. it represents by processing raw material containing 0·18 per cent. of morphine instead of 0·20 per cent. In variety B it would be necessary to harvest the top half of the plant to recover all the morphine. The concentration of the alkaloid would then be almost half that of the husks alone, and in this case it is doubtful if it would be worth while recovering all the morphine. Possibly the most economical point of cut would be somewhere in the third quarter.

Certain varieties have a number of side branches, the capsules of which are below that on the main stem. In such cases, it would appear desirable to make the cut sufficiently low to include the majority of the capsules. In view of variations due to seasonal and varietal differences which will undoubtedly be encountered in practice, it is suggested that the cutter bar of the header be set not lower than is necessary to take off the bottom capsules nor higher than three-quarters of the average height of the crop.

## 5. Conclusions.

1. Under Australian Capital Territory conditions the husks of the opium poppy increased in dry weight during the first two weeks after petal fall, but decreased about 10 per cent. during the third and fourth weeks.

2. The dry weight of the stems decreased throughout the maturation period, the top and bottom portions decreasing in approximately the same proportion. The top half contained only about one-fifth of the total dry matter of the stems.

3. The seeds reached their maximum weight about three weeks after petal fall.

4. The percentage of morphine in the dry matter of the husks was at a maximum at petal fall, but did not vary substantially from the 14th to the 35th day. The absolute weight of morphine reached a maximum sometime after the 14th day after petal fall, the exact stage being dependent upon seasonal conditions and upon the variety.

5. Morphine was concentrated in the upper half of the stem, and decreased approximately in geometric progression in successive sections from the base of the capsule downwards.

6. Neither leaves nor roots contained measurable amounts of morphine during the second half of the maturation period.

7. It is recommended that the capsules and the upper portion of the stems only should be harvested. The cutter bar should be set low enough to include the majority of the capsules and not higher than three-quarters of the average height of the stems. The crop should be harvested as soon as the capsules are dry enough to bag directly from the header.

## 6. Acknowledgments.

The author wishes to thank Mr. G. A. McIntyre for help in the mathematical treatment of the data, and the farm manager, Mr. L. R. Sharp, for supervizing the spray irrigation experiment.

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# Microphotography as an Aid to the Identification of Trombiculine Larvae.

*D. A. Gill, M.R.C.V.S., D.V.S.M.,* and E. Parrish.**

*Summary.*

The difficulty of identifying somewhat similar species of trombiculine mites from descriptions, drawings, and measurements is discussed. The use of microphotography of the whole mite and of its dorsal scutum is recommended as an additional aid.

The photographic technique used to obtain the accompanying illustrations is given in detail.

It is considered that a complete series of such photographs, covering all the known species of trombiculine mites, would be of great assistance.

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Many new species of trombiculine larvae have been examined by different workers recently, more especially since bush typhus has been the subject of intensive investigation in New Guinea. Womersley and Heaslip (1943) and Womersley (1944) in particular have made a detailed study of these parasites in Australia and Oceania. When there are striking differences between species it is not difficult to identify them, but in many cases the differences are not particularly striking. The differentiation of one species from a closely similar one has depended on descriptions and drawings or else on measurements which themselves show a considerable range within a given species and often overlap with those of specimens which have been allocated to other species.

Radford (1942) has published a descriptive key and included drawings of the dorsal scutum of each species, but he gave no measurements and the drawings by themselves are insufficient.

In the absence of some other consistent and obvious characteristic, Womersley and Heaslip (loc. cit.) differentiate largely on differences in the size, shape, and proportions of the dorsal scutum and its appendages as determined by actual measurement, and they have achieved a great deal by so doing. They say: "The careful measurement of a standard series of data from the scutum of the different species and a comparison of the arrangements of the dorsal setae have shown that these can be used to distinguish specifically the many species of larvae."

Nevertheless, even with the aid of Womersley and Heaslip's published keys, which include measurements and camera lucida drawings, it is often a very difficult matter for other workers to decide whether specimens which they find in the field are new, or belong to a species already described. Lists of measurements do not enable one to visualize the appearance of the scutum they concern, and descriptions and drawings, unless they show striking differences, are often insufficient for this purpose. Measurements may be misleading. Setae or sensillae may be bent or broken so that the measurement is difficult, or they may be fractured diagonally along their length and pulled out so as to seem longer than they really are; as the mite engorges the swelling of its

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body tips the dorsal scutum forward so that it is foreshortened and hence, although the scutum is not altered by engorgement, its measurement may be inaccurate in recently mounted specimens.

In view of these difficulties, which all workers in this field must have encountered, we believe that much would be gained both in time and precision if photographs were available with which field workers and others could compare specimens. If the species already known were photographed, the series could be kept up to date as others were discovered.

Our experience in this field is limited, but we have found that microphotographs of dorsal scuta show their details with great clarity. In fact, with the technique we have used, the photograph *taken by oblique illumination* often shows details more clearly than they can be seen through the microscope. For example, it is often extremely difficult to discern through a microscope the anterior border of the scutum in *Leeuwenhoekia,* but reference to Plates 3 and 4 will show how clearly it is delineated therein. They also show two small pits, regularly placed, near the postero-lateral setae in both species, which have not been noticed hitherto.

To indicate the value of microphotography as an adjunct to other means of identification, we produce here photographs of two species of *Trombicula* (Plates 1 and 2), two of *Leeuwenhoekia* (Plates 3 and 4), and two of *Paraschongastia* (Plates 5 and 6). These specimens were all collected recently from animals and birds in Australia.

Provided suitable microphotographic apparatus is available, it is not a difficult matter to make such photographs. It is, of course, essential that precisely the same magnification be used throughout and that the specimens photographed be as perfect as possible and well mounted. For the photograph of the mite itself it is highly desirable to secure a specimen which has not yet attached to its host and commenced to feed, otherwise allowance must be made for the state of engorgement in judging what the unengorged mite would look like. This is obviously unsatisfactory, but a partially engorged mite is better than nothing, since its photograph will at least show the arrangement of dorsal setae and various other features which will assist in differentiation.

The use of microphotography could be carried farther and all differences between the many species could be charted with its aid, but it is at least doubtful whether this would be justified except in a few special cases. Other differences which are used in differentiation usually do not depend so much on appearance or measurements; they are features which either are, or are not, present, and hence there is no difficulty in recording them in written keys.

## The Photography of the Mite.

A 16 mm. achromatic objective with an N.A. of 0·25 and an initial magnification of 10 × was used with a 7 × compensating ocular. Anything lower than this resulted in insufficient resolution of detail, while an objective with a higher N.A. gave insufficient depth of field to bring all parts of the specimen into reasonably sharp focus without undue restriction of the aperture.

The mounting of the specimen plays an important part in the success of the photograph. Care should be taken to see that a minimum of mountant is used and is allowed to dry out somewhat, otherwise it will be found impossible to bring the dorsal surface and the extremities into sharp focus at the same time. Focusing is critical, and a focal plane should be chosen which shows the details of the dorsal surface and extremities at their best, while subduing as much as possible the setae and structure of the ventral surface. In this last, the degree of restriction of the N.A. plays an important part. Wratten filters, B + H, were used to increase contrast within the object itself.

Strong contrast plates such as Kodak Extra Rapid Process Pan, or Process Pan, fully exposed and developed for two-thirds of the time in D 19 have given the best results.

## The Photography of the Scutum.

An 8 mm. apochromatic objective with an N.A. of 0·65 and an initial magnification of 20 × with a 10 × compensating ocular was used. Oblique illumination has been found to give far superior results to that obtained with axial light. It helps to delineate the margins of the shield more clearly and throws into strong relief the texture and structure of the scutum generally. Too great obliquity of light must be avoided, otherwise shadow formation will be too strong. The direction of the oblique beam must also be controlled by revolving the stage until the best result is obtained. This is usually when the beam strikes one side and the anterior margin of the scutum, but the actual angle needs some adjustment from specimen to specimen.

Stopping down for depth of focus should be watched carefully to see that the shadows of underlying structures do not become too prominent, but this again varies from one specimen to another. Less restriction of aperture can be used with oblique illumination than with axial lighting.

For *Trombicula* and *Leeuwenhoekia* a Wratten C filter gives the best results, but for the *Paraschongastia,* particularly if they have been mounted in polyvinyl alcohol coloured with picric acid, it is better to move up the wave band a little and use an H or B. The choice of filter and of all optical adjustments is best done while observing the object from the microscope, and not from the screen, using a high power ocular which can afterwards be replaced with the lower powered ocular with which the photograph is to be taken.

Strong contrast plates, such as Extra Rapid Process Panchromatic, should be used, and exposure and development adjusted according to the requirements of the specimen.

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# The Use of D.D.T. as an Agricultural Insecticide.

## Results of Trials, 1944-45.

*By G. A. H. Helson, M.Sc.,* and T. Greaves.**

*Summary.*

The results of trials on the use of D.D.T. showed that it gave effective control of a number of pests, e.g., green vegetable bug, *Nezara viridula* L.; bean aphid, *Doralis fabae* Scop.; black peach aphid, *Anuraphis persicae-niger* Sm.; green peach aphid, *Myzus persicae* Sulz.; potato aphid, *Macrosiphum gei* Koch; Oriental peach moth, *Cydia molesta* Busck.; codling moth, *Cydia pomonella* L.; potato moth, *Gnorimoschema operculella* (Zell.); cabbage centre grub, *Hellula undalis* (Fabr.); cabbage cluster grub, *Crocidolomia binotalis* Zell.; common cluster grub, *Prodenia litura* Fabr.; corn earworm, *Heliothis armigera* Hubn.; cabbage moth, *Plutella maculipennis* Curtis; cabbage butterfly, *Pieris rapae* L.; pear slug, *Caliroa limacina* de Geer; and certain ants. This list contains certain species which could not be controlled adequately by insecticides used previously. On the other hand, a few pests proved to be resistant to D.D.T. in the preparations used, notably cabbage aphid, *Brevicoryne brassicae* L.; woolly aphid, *Eriosoma lanigerum* Hausm.; and red spider, *Tetranychus urticae* Koch.

### I. Introduction.

Early in December 1943, a small amount of D.D.T. was obtained for the study of its effectiveness against crop pests. Since then, larger amounts have been obtained for the same purpose, and some large-scale field trials have now been made.

In July 1944, a conference was convened in Melbourne by the Commonwealth Food Control, Commonwealth Department of Commerce and Agriculture, to consider what steps should be taken to test the usefulness of D.D.T. as a substitute for nicotine sulphate for the control of agricultural and horticultural pests. This Conference considered that until commercial quantities of D.D.T. were available, any supplies surplus to Army requirements should be used, as far as possible, for experimental work designed to determine the potentialities of D.D.T. as an insecticide against pests of vegetable and horticultural crops in Australia. It was suggested that all State Departments of Agriculture, as well as the Waite Agricultural Research Institute, Adelaide, and the C.S.I.R., should co-operate in the experimental work needed, and that some co-ordination of the programme throughout the Commonwealth would be desirable.

After this, a co-operative programme of work was agreed upon between the State Departments of Agriculture and the C.S.I.R., and a conference to discuss the results of the season's investigations on the use of D.D.T. against agricultural pests was held in Canberra from June 4 to 8, 1945. These will be published, for the most part, in the various State agricultural journals. The present article deals with the results obtained by the C.S.I.R. up to July, 1945.

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### 2. Forms in which D.D.T. was applied.

A review of overseas reports indicated that D.D.T. dusts were suitable for application to crops, and that there were a number of emulsions, some of which could be used as sprays. The solvents, and some of the emulsifiers used in the sprays, were not available in Australia, so it was necessary to find a suitable mixture to form the basis of experiments during the 1944-45 season. It was decided to use a mixture containing 90 per cent. pyrophyllite and 10 per cent. D.D.T., and this was coloured bright-blue to facilitate the uniform mixing of dilute dusts. This 10 per cent. D.D.T. dust could also be used as an aqueous suspension for application of D.D.T. as a spray.

A solvent naphtha emulsion was evolved during the course of laboratory work on the control of potato moth *Gnorimoschema operculella* (Zell.) at Canberra, A.C.T. Tests on potato plants using some coal tar derivatives had already shown that solvent naphtha was the least phytocidal of these (Helson and Powning, unpublished data). Further investigation showed that it would dissolve at least 20 per cent. D.D.T. at ordinary temperatures, and that a very stable emulsion could be made when suitable emulsifying agents were used.

The solvent naphtha emulsion is prepared as follows:—A stock solution is made by dissolving 1 lb. of D.D.T. in 5 pints of solvent naphtha (boiling range of 90-190°C.) and adding to this half a pint of an emulsifier (the sodium salt of alkyl naphthalene sulphonic acid, and pine oil). This quantity of stock solution, when mixed with water to make 100 gallons of spray, produces an emulsion containing 0·1 per cent. D.D.T. Solvent naphtha obtained by distillation from coal tar is very variable in composition, and is not a suitable solvent for the preparation of a uniform emulsion. It is probable, therefore, that it will be replaced by other less variable materials.

Attempts to incorporate D.D.T. in some white oil emulsions have not been satisfactory, since the conventional petroleum oils seldom dissolve more than 5 per cent. D.D.T. When these white oils containing D.D.T. are diluted to the final spray strength, it is often found that D.D.T. is thrown out of solution and adheres to the sides of the containing vessel.

Emulsions have also been prepared using eucalyptus oils as the solvent for D.D.T. (Plante, unpublished data). These oils dissolve 25 per cent. D.D.T. at room temperatures, form very stable emulsions with many emulsifying agents, and sprays made from the stock emulsions appear quite satisfactory.

The question of finding the best formulation for the preparation of stock D.D.T. emulsions is still being investigated, and several promising mixtures are being considered. In the meantime, extensive field work has been carried out using the D.D.T. solvent naphtha emulsion, and this formulation will continue to be used until a better one is evolved.

### 3. Phytotoxicity Tests.

Dusts containing D.D.T. have been found quite safe to use on crops. Phytotoxicity tests were made first with the D.D.T. solvent naphtha emulsion on potato plants, and a concentration of 0·1 per cent. w/v D.D.T. emulsion was found to be non-injurious. Tests were also made

at Home Hill, North Queensland, with a 0·2 per cent. D.D.T. emulsion on the following crops without any apparent injury: beetroot, egg plant, and rhubarb; seedlings of French bean (Brown Beauty), capsicum, tomato, lettuce, passion fruit, and cabbage. Cucumber seedlings, however, were seriously affected, and lucerne and papaw seedlings were slightly affected.

Phytotoxicity tests were also carried out at Canberra with some white oil D.D.T. emulsions, the concentration of D.D.T. in the final spray mixture being 0·1 per cent. The most marked effect of these emulsions was the inhibition of growth on potted French bean seedlings as shown by leaf measurements, i.e., length of the mid-rib, greatest breadth of the leaflet, and length of the internode between this leaf and the next pair of leaves. Measurements were made immediately prior to spraying, and again one week later. They showed that the D.D.T. white oil emulsions used inhibited the growth of bean plants under glasshouse conditions. The leaves of treated plants did not expand to a size more than half that of untreated plants, the flowers were smaller, and the length of the internodes greatly reduced. In these trials, the same white oil emulsions, without D.D.T., did not produce any inhibitions of plant growth. The degree of inhibition of plants treated with different D.D.T. white oil emulsions varied considerably, and some inhibition of growth also occurred with D.D.T. eucalyptus oil emulsions. Solvent naphtha D.D.T. emulsion caused slight inhibition of growth of beans, but this was less than that produced by any other D.D.T. emulsion.

In field trials in North Queensland, over 600 gallons of solvent naphtha D.D.T. emulsion were applied to potatoes without any injurious effect, and the experimental potato crop at Dickson, A.C.T., was sprayed three times with 0·1 per cent. D.D.T. emulsion without any injury. In the autumn of 1945, all plants in one glasshouse at Canberra were sprayed with a 0·1 per cent. D.D.T. emulsion for the control of potato moth and other insects which were infesting plants being grown for experimental work. A large variety of plants was growing in the glasshouse at the time, including many young seedlings. The only phytotoxicity noticed was a very slight scorching on the tips of young *Schizanthus* sp. seedlings which were just coming up, and possibly very slight injury on a few very young potted potato seedlings. All the results to date suggest that the solvent naphtha D.D.T. emulsion is quite safe to use on crops.

### 4. Results of Experiments.

(i) *Hemiptera.*

(*a*) Nysius vinitor *Berg.* (*Rutherglen Bug.*)

Preliminary laboratory experiments with 1 per cent. D.D.T. dust gave 100 per cent. mortality of adult bugs in 17 hours.

(*b*) Nezara viridula *L.* (*Green Vegetable Bug.*)

In the first experiments, individual adult insects were fastened to micro slides with paraffin wax, and technical grade D.D.T. crystals were dusted on to various portions of the body. These included tarsi only, thorax, abdomen, entire dorsal surface, including the hemelytera, and the antennae only. Within 15 minutes all bugs, except those dusted on the dorsal surface, showed signs of irritation and excitation. The period of excitation was followed by a prolonged period of slowly

decreasing activity, and paralysis of the limbs. The insects then passed into a state of coma, and finally, death ensued. Bugs whose antennae were dusted succumbed first, followed by those whose tarsi had been treated. The last to die were bugs whose thorax and abdomen had been dusted. The control bugs were alive at the end of the experiment. Similarly, bugs whose antennae had been treated with a small amount of a supernatant mixture of D.D.T. and distilled water, all died.

Tests were then made, using a standard dusting apparatus for the application of dusts containing 0·1, 0·25, 0·5, 1, 3, and 5 per cent. D.D.T. in kaolin. In this case, the dusts were applied to ripe tomatoes artificially infested with all stages of the bug. Each of the dusts killed all stages in from 36-48 hours, most of them being effective in 24 hours. Controls were all alive at the end of this time.

The first small-scale field test at Canberra in March, 1944, was made using large gauze cages to confine bugs on dusted tomato plants. The plants were dusted with 5 per cent. D.D.T. in kaolin and all stages of the bug were released on the plants. At the end of one week, the treatment had produced 64 per cent. mortality.

Several small tests were made in Canberra home gardens during February and March, 1945. Four plots of tomatoes, two staked and two dwarf type, and one plot of French beans, all infested with *N. viridula,* were dusted with 1 per cent. D.D.T.; in addition, one plot of infested tomatoes was sprayed with 0·1 per cent. D.D.T. solvent naphtha spray. The treatment in all cases gave a complete control of the bugs on the treated plants, and caused the bugs to leave the untreated plants in adjacent rows; a single application gave protection for one month. In one untreated plot, situated away from the dusted plot in the same garden, the number of bugs increased during the test period.

(ii) *Aphididae.*

(*a*) Doralis fabae *Scop.* (*Bean Aphid*).

A small experiment was made on a row of broad beans badly infested with bean aphid. The plants were sprayed with 0·1 per cent. D.D.T. solvent naphtha emulsion, and 100 per cent. kill was obtained after a single application. It was observed that bees visiting the flowers while still wet with spray were seriously affected by the D.D.T.

(*b*) Anuraphis persicae-niger *Smith* (*Black Peach Aphid*).

Seven peach trees severely affected by the black peach aphid were sprayed with 0·1 per cent. D.D.T. solvent naphtha emulsion. At the end of one week, the spray had produced at least 90 per cent. kill, the trees were recovering from the attack, new growth remained free from attack, and no leaf injury was observed. Only one spray was applied, and this afforded sufficient protection to the trees until adverse weather conditions killed the remaining aphids and thus permitted the normal growth of new shoots.

(*c*) Brevicoryne brassicae *L.* (*Cabbage Aphid*).

Early laboratory experiments with 5 per cent. D.D.T. dust in kaolin suggested that this dust was effective against cabbage aphid. Subsequently, however, in large field trials on cabbages at Fyshwick, A.C.T., it was found that 0·5 and 1 per cent. D.D.T. dusts at 20 lb. per acre, or 0·5 and 0·1 per cent. D.D.T. solvent naphtha emulsions at 80 gallons per acre, did not satisfactorily control cabbage aphid.

(*d*) Eriosoma lanigerum *Hausm.* (*Woolly Aphid*).

Two applications of 0·1 per cent. D.D.T. solvent naphtha emulsion were made at the rate of 2 gallons per tree, at an interval of one month, to badly affected apple trees in an orchard at Weetangerra, A.C.T. The treatments were not effective against the aphids.

(*e*) Myzus persicae *Sulz.* (*Green Peach Aphid*), *and* Macrosiphum gei *Koch* (= solanifolii *Ashm.*) (*Potato Aphid*).

Laboratory trials with 5 per cent. D.D.T in kaolin gave 100 per cent. mortality in 48 hours.

Early in the spring of 1944, three-quarters of an acre of lettuces became severely infested with *M. persicae* and *M. gei,* at Fyshwick, A.C.T. The plot was dusted with talc impregnated with 2 per cent. D.D.T., diluted with equal parts of pyrophyllite to give a 1 per cent. D.D.T. mixture. A wheelbarrow Root power duster was used to apply the dust at the rate of 32 lb. per acre. At the end of two days, this treatment produced 68 per cent. mortality, and 88 per cent. mortality at the end of the week. No further dustings were required, and no plant injury resulted.

In a large field trial at Home Hill, North Queensland, 1 and 5 per cent. D.D.T. in pyrophyllite at 35 lb. per acre gave complete control of *M. persicae* seriously affecting a late crop of cabbages, and 0·1 per cent. D.D.T. solvent naphtha emulsion gave complete control of the same pest on potato crops.

(iii) *Lepidoptera.*

(*a*) Cydia molesta *Busck.* (*Oriental Peach Moth*).

A small field experiment was made in Ardmona, Victoria, on a block of 36 young Pullar Cling peach trees in the first year of bearing. Two applications of 0·1 per cent. D.D.T. solvent naphtha emulsion were made late in the season with the object of protecting the fruit from attack by Oriental peach moth. The first spray was applied on February 6, 1945, and the second on February 21. The spray was applied at the rate of approximately 2 gallons per tree. Each tree was surrounded by one row of buffer trees, and one half the 36 trees in the experiment were sprayed on a randomized plan, and the other eighteen left untreated as controls. The figures at harvest are shown in Table 1.

Table 1.

| — | Number of Fruit on Treated Trees. | Number of Fruit on Untreated Trees. |
|---|---|---|
| Sound fruit .. .. .. | 1,949 | 1,659 |
| Moth infested fruit .. .. .. | 73 | 116 |
| Total fruit .. .. | 2,022 | 1,775 |
| Percentage infestation .. | 3·6 | 6·5 |

Analysis of the fruit at harvest showed that there was a D.D.T. residue of approximately 9 p.p.m. A portion of the treated crop was canned in the normal way, and an analysis of the canned fruit showed that no D.D.T. was present (Powning, unpublished data).

(*b*) Cydia pomonella *L.* (*Codling Moth*).

A small field trial was carried out with D.D.T., among other treatments, on single tree plots on a block of 17 pear trees in the experimental orchard at Canberra, A.C.T. 0·1 per cent. D.D.T. solvent naphtha emulsion was applied on an ordinary lead arsenate schedule and was compared with standard lead arsenate spray. The petal fall spray was applied on October 13, 1944, and was followed by four subsequent applications. At harvest, 100 per cent. clean fruit was picked off the variety William Bon Chretien, and very little fruit had fallen to the ground at this time. If the figures for the dropped fruit are included, then the percentage of sound fruit at harvest was 95 per cent. The fruit on the control trees was 100 per cent. infested, and a large part of the crop had dropped to the ground at harvest.

Another small experiment was made in the Goulburn Valley, Victoria, in February, 1945, on part of a block of young Pakenham pears in the first year of bearing. Every second pair of trees in the first four rows of the block was treated with D.D.T., and every tree in the fifth row sprayed. All trees in the sixth row were left as controls for comparison with row 5; there were 35 trees to a row. These trees had received the ordinary lead arsenate schedule up till the end of December, 1944, after which no further treatment had been made. A single spray of 0·1 per cent. D.D.T. emulsion, at the rate of approximately 2 gallons per tree, was applied on February 6, 1945, at which time approximately 5 per cent of fruit was infested and third generation codling moth eggs were being deposited. The fruit in the first four rows was harvested on February 19, 13 days after the spray application, and at this time 14 cases of sound fruit and 1·25 cases of infested fruit were harvested from the trees sprayed with D.D.T.; 12 cases of sound, and 1·33 cases of infested fruit were harvested from the unsprayed trees. Thus, infestation on the D.D.T.-treated trees was 8 per cent. and that on the untreated trees 10 per cent. These figures do not take into consideration the initial infestation. Rows 5 and 6 were harvested on February 26, when 11 cases of sound fruit and 1·33 cases of infested fruit were harvested from the D.D.T.-treated plots, and 7·5 cases of sound and 2·5 cases of infested fruit from the control row. This represents 10·5 per cent. codling moth infestation in the D.D.T.-treated plots and 25 per cent. in the control. Thus, three weeks after application, D.D.T. had reduced infestation of the fruit by codling moth. The foregoing results indicate that D.D.T. is a very promising material for the control of codling moth. Some satisfactory means of controlling mites will be necessary, however, in areas where these pests are prevalent, if overseas reports of heavy mite infestation following the use of D.D.T. are correct.

(*c*) Gnorimoschema operculella (*Zell.*) (*Potato Moth*).

Laboratory tests have been carried out against the larvae of the potato moth with both dusts and sprays. In the earliest experiments, 5 per cent. D.D.T. mixed with kaolin was applied with a standard dusting apparatus to larvae of the potato moth. This dust produced 100 per cent. mortality 40-46 hours after application.

Tests were also made on the effect of dusts on adult potato moths. 1 per cent. D.D.T. was applied with a standard dusting apparatus to leaves of potato plants, and adult moths were then placed in jars with the dusted potato plants. At the end of 72 hours, the 1 per cent. D.D.T. dust had produced a mortality of 83 per cent. in the first test, and a mortality of 85 per cent. in the second (percentages determined by Abbott's (1925) formula). These small-scale laboratory tests suggest that D.D.T. is toxic to adult potato moths. Laboratory spray trials were also made, using D.D.T. solvent naphtha emulsions at concentrations of 0·1, 0·5, 1, and 2 per cent. on newly hatched potato larvae placed on potato plugs. Results at the end of nine days are shown below:—

| Percentage concentration | 0·1 | 0·5 | 1 | 2 |
|---|---|---|---|---|
| Average percentage mortality | 96·4 | 91·8 | 89·2 | 87·5 |

Thus, in this test, the higher concentrations of D.D.T. were not as toxic as the lower concentrations.

Large-scale field experiments were carried out on potato crops at Home Hill, North Queensland. A 0·1 per cent. D.D.T. solvent naphtha, at the rate of 110-120 gallons per acre, was applied to three different crops maturing as early, mid-season, and late crops. Very little moth infestation occurred in the early and middle season crops, and the spray gave the greatest protection to the tubers of the late crop, the haulms of which were affected by target spot (*Alternaria solani*).

A field test with 0·1 and 0·2 per cent. D.D.T. solvent naphtha sprays at the rate of 80 gallons per acre was made on a severe infestation of potato moth on 300 egg plants in the same district. At the end of three days, 100 per cent. mortality was produced by both treatments.

At Dickson, A.C.T., 1 per cent. D.D.T. dust, at 40 lb. to the acre, and 0·1 per cent. D.D.T. solvent naphtha emulsion, at the rate of 110-120 gallons per acre, were included among other treatments in a large field test. This crop was severely attacked by blight late in the season, and no observations on the direct effect of D.D.T. for the control of potato moth in the growing haulms could be made. At harvest, the only plots which showed a greater yield of sound potatoes were those treated with 0·1 per cent. D.D.T. solvent naphtha spray.

A large field dusting trial was made at Crookwell, N.S.W., where three applications of 0·5 per cent. D.D.T. dust and 20 per cent. cryolite dust were made at the rate of 60 lb. to the acre on a crop of 3 acres of potatoes. The first application was made on January 26, 1945, the second on February 22, and the third on March 19. On February 22, the crop was rated for damage by potato moth to the haulms by a method described by Bald and Helson (1944). The following were the results: D.D.T. 0·68, cryolite 0·82, and control 0·92. These figures represented the average rating for damage per plant. At this date, there was no significant difference between cryolite and control, but D.D.T. was significantly better than both of these treatments. No further ratings were possible on this crop because early frosts intervened. At harvest in May, the average yield of sound tubers per plant was 0·9 lb. in untreated plots, 1·1 lb. in cryolite plots, and 1·3 lb. in D.D.T. plots. Infestation was 9 per cent. in control, 11 per cent. in cryolite, and 9 per cent. in D.D.T. plots. Thus, in this experiment, D.D.T. did not give protection to the tubers in the ground. Increased yield of sound tubers resulted, however, from the protection of the

haulms with D.D.T., and the difference was just significant at the 5 per cent. level. There was no significant difference between control and cryolite. The increase in yield per acre on the D.D.T.-treated plots was 0·7 ton.

One other large field trial was carried out in the Batlow district of New South Wales. A portion of the crop was treated with a single application of 1 per cent. D.D.T. dust, applied at the rate of 30 lb. to the acre in the third week in March. The plots treated with D.D.T. were a portion of an area which had been treated previously, about a month before, partly with derris and partly with cryolite. At harvest in May, the percentage of moth damage in these plots was as follows: derris 4·5 per cent., derris followed by D.D.T. 0·8 per cent., cryolite 1·8 per cent., cryolite followed by D.D.T. 0·4 per cent., control 13·2 per cent.

There is now sufficient evidence, including unpublished data, to show that 0·1 per cent. D.D.T. solvent naphtha emulsion, at the rate of 110-120 gallons per acre, or 2 per cent. D.D.T. dust, at the rate of 30-40 lb. per acre, should give adequate control of the potato moth in the growing crop. Large field tests with D.D.T. in a number of different districts are planned for next season.

(*a*) Hellula undalis (*Fabr.*) (*Cabbage Centre Grub*), Crocidolomia binotalis *Zell.* (*Cabbage Cluster Grub*), Prodenia litura *Fabr.* (*Common Cluster Grub*), Heliothis armigera *Hubn.* (*Corn Earworm*).

In a field experiment, carried out during June and July, 1944, on an early crop of cabbages in the Burdekin area, North Queensland, D.D.T. dusts (1 and 5 per cent.) gave nearly complete control of the cabbage centre grub, cabbage cluster grub, common cluster grub, and corn earworm, after four applications, each at 10 days' interval (Greaves, 1945).

(*e*) Plutella maculipennis *Curtis* (*Cabbage Moth*).

In a second field experiment on a late crop of cabbages in the Burdekin area, *P. maculipennis* and *M. persicae* were the dominant pests and in a test involving 30 treatments, the D.D.T. dusts (1 and 5 per cent.) were significantly better than all other treatments.

At Fyshwick, A.C.T., two additional field experiments were carried out. In the first, a preliminary test on a spring crop of cabbages, maturing late in December, 1944, D.D.T. solvent naphtha sprays (0·05 and 0·1 per cent. D.D.T.) and D.D.T. dusts (0·5 and 1 per cent.) gave nearly complete control and were significantly superior to the 20 per cent. lead arsenate plus 6 per cent. nicotine sulphate dust after two treatments at 12 days' interval.

A more detailed study of D.D.T. dusts and sprays was carried out in February-March, 1945, on a late crop of 5,200 cabbages situated between two similar crops that were heavily infested with *P. maculipennis*. Included in this experiment were D.D.T. dusts (0·5 and 1 per cent.) applied at 20 lb. to the acre at 10-day and 14-day intervals, and D.D.T. solvent naphtha sprays (0·05 and 0·1 per cent. D.D.T.) at 80 gallons per acre, also applied at 10-day and 14-day intervals.

All the D.D.T. treatments gave nearly complete control of the cabbage moth, and were outstanding when compared with the 20 per cent. lead arsenate plus 6 per cent. nicotine sulphate dust treatment at 10-day intervals.

The D.D.T. sprays (0·05 and 0·1 per cent) were significantly better than the D.D.T. dusts (0·5 and 1 per cent.) when applied at either 10-day or 14-day intervals. There was no significant difference between the same D.D.T. dusts applied at intervals of 10 days or 14 days, but the 1 per cent. D.D.T. dust applied at 10-day intervals was significantly better than the 0·5 per cent. D.D.T. dust applied at 14-day intervals. The 0·1 per cent. D.D.T. spray was significantly better than the 0·05 per cent. D.D.T. spray when applied at both 10-day and 14-day intervals.

(*f*) Pieris rapae *L.* (*Cabbage Butterfly*).

Larvae of the cabbage butterfly were present in the crops at Fyshwick, A.C.T., and were controlled by all D.D.T. treatments used (see above). No living larvae were seen after the first application of D.D.T.

(iv) *Hymenoptera.*

(*a*) Caliroa limacina *de Geer* (*Pear Slug*).

A single application of 0·1 per cent. D.D.T. solvent naphtha emulsion gave complete control of pear slug on a William Bon Chretien pear tree. The trial was repeated using 0·05 per cent. emulsion; this also gave complete control of the pest.

(*b*) Iridomyrmex detectus (*Sm.*) (*Meat Ant*).

Sixteen colonies of the meat ant were treated at Canberra, A.C.T., in October, 1944, with 5 per cent. D.D.T. dust at the rate of 1 oz. to every four entrance holes; three colonies were treated in December, 1944, with 1 per cent. D.D.T. dust at the rate of 1 oz. to every five entrance holes, and one colony was treated in January, 1945, with two quarts of 0·1 per cent. D.D.T. solvent naphtha emulsion. All the colonies were completely controlled within one week of treatment, but in each case the treatment did not prevent the re-population of the nest sites by connected or neighbouring colonies.

(*c*) Technomyrmex albipes (*Sm.*) (*Black House Ant*).

Five per cent. D.D.T. dust, dusted into cupboards, and into a nest of this species, at Canberra, A.C.T., gave complete control.

(*d*) Pheidole megacephala (*Fabr.*) (*Brown House Ant*).

Five per cent. D.D.T. dust, dusted on floors of offices and in shallow tins around the legs of a refrigerator at Home Hill, North Queensland, gave complete control of the brown house ant.

(v) *Acarina.*

Tetranychus urticae *Koch* (*Red Spider*).

Bean plants growing in the glasshouse became severely infested with this mite, and were sprayed with 0·1 per cent. D.D.T. solvent naphtha. The spray was ineffective.

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# Differential Isolation of *Chaetomium* Spp. from Mixed Populations by Hypochlorite Solution.

*By D. O. Norris, M.Sc. (Agric.)**

*Summary.*

Spores of the fungal genus *Chaetomium* are highly resistant to calcium hypochlorite solution which is a very efficient sterilizing agent against other organisms. Large numbers of spores of *C. globosum* can survive immersion for fifteen minutes, and an occasional spore may survive immersion for up to two hours, in a solution containing approximately 2 per cent. chlorine. This differential sensitivity enables *Chaetomium* spp. to be isolated at will from mixed populations.

## 1. Introduction.

For some time extensive use has been made in this laboratory of calcium hypochlorite solution as a surface-sterilizing agent during routine isolation of pathogens from diseased plant material. The effectiveness of this method of surface sterilization has been known for 30 years, since the original work of Wilson (1915), and pathologists have used it in various ways (Knudson, 1929 and 1933; Gordon, 1937; White, 1938; Wilson and Punyasingha, 1939). In the work here described, the method of preparation originally set out by Wilson was used. Ten grams of ordinary commercial bleaching powder was shaken with 140 cc. of tap water for one minute and filtered to yield a pale-yellow solution smelling strongly of chlorine. Such a preparation was stated by Wilson to contain about 2 per cent. chlorine in solution. All material to be surface-sterilized was first rinsed in 70 per cent. alcohol, then immersed in the hypochlorite solution for five minutes and plated without washing. Thus the pieces of tissue remained saturated with hypochlorite solution for some time after plating. Plates used were always ordinary petri dishes, 10 cm. by 2 cm., and the medium potato-dextrose agar.

Over a long period of time, it was noticed that, provided four or five pieces of tissue were placed in a plate, aerial contamination with bacteria and moulds of the *Penicillium* type was completely eliminated. It became apparent that gaseous chlorine diffusing through the air inside the closed plate from the saturated pieces of tissue was killing all bacteria and fungal spores that had entered during plating operations. Comparative trials were made of this method against the usual 1/1000 mercuric chloride followed by several washings with sterile water. Air turbulence was deliberately created during the platings to increase the chances of aerial contamination. Mixed contaminations regularly developed on the plates of the mercuric chloride series, but were absent from the hypochlorite series with the exception of an occasional *Chaetomium* colony.

At the same time as the absence of aerial contaminations became noticeable, it was observed that species of *Chaetomium* were isolated with much greater frequency from tissue sterilized by the hypochlorite method than from tissue sterilized with mercuric chloride. Several theories to explain this were tested, and finally all evidence pointed

* An officer of the Division of Plant Industry.

to the fact that spores of species of *Chaetomium* were very resistant to hypochlorite and were not killed by five minutes' exposure to the solution, nor by the exposure to gaseous chlorine subsequent to plating.

### 2. Experimental.

A series of sixteen plates was exposed for five minutes on the laboratory floor, during which time dust was stirred up to produce contamination, and, in addition, a culture of a common green *Penicillium* was blown over the plates. To eight plates were then added five small blocks of potato tissue as carriers of hypochlorite solution, and all plates were incubated for three days at 25°C. By this time, untreated plates were completely covered with thousands of colonies of *Penicillium*, as well as bacteria and other fungi such as *Alternaria* spp. The treated plates at first appeared sterile, but after several more days' incubation scattered colonies of *Chaetomium* spp. appeared. When these were approximately half an inch in diameter, two of the plates were treated again by rewetting the pieces of tissue with hypochlorite. The *Chaetomium* colonies were apparently not affected, growing on without any sign of retardation.

Subsequent tests were made with uniform solid glass beads approximately 6 mm. diameter in place of the pieces of plant tissue of variable size and shape previously in use. These beads carried approximately equal quantities of hypochlorite solution on their surfaces when placed on the agar with forceps. Several experiments were done using these beads with essentially similar results, but only two will be described. In the first of these, 40 plates were exposed on the laboratory floor for half an hour, during which time attempts were made to produce maximum contamination. Dust was beaten up from the floor and blown from shelves on to the plates. At the end of this time, 20 plates were closed as controls. The others were divided into four lots of five on which were placed 4, 6, 8, and 10 beads of hypochlorite respectively. After six days' incubation at 25°C. the results were as shown in Plate 7.

The control plates were covered with a mass of colonies of bacteria and miscellaneous fungi such as *Rhizopus, Mucor, Penicillium,* and *Alternaria.* The plates bearing the beads showed only pure cultures of *Chaetomium* spp. On the four-bead series, a few cultures of fungi other than *Chaetomium* appeared, but on all the others cultures were exclusively *Chaetomium.* The number of beads did not affect the number of *Chaetomium* colonies.

In the second experiment, fifteen plates were exposed for one hour on the floor and contaminated as before; five plates were left as controls, five were treated with four beads each, and five with 25 beads each. The interesting result shown in Plate 8, Fig. 1, was obtained. The control plates were completely covered with miscellaneous colonies as before, but in this instance, probably owing to variation in quality of the bleaching powder used, four beads were insufficient to inhibit fungal colonies although bacteria were completely eliminated. As shown in the figure, *Chaetomium* spp. predominated, but also present were *Rhizopus, Stemphylium, Alternaria,* and a few colonies of *Penicillium* spp. A concentration of hypochlorite represented by 25 beads did not inhibit the growth of *Chaetomium* spp., the five plates bearing an average of ten colonies.

To test the resistance of spores to the actual hypochlorite solution, a suspension of spores of *Chaetomium globosum* was obtained by picking the spore horns from the perithecia with a needle and dispersing them in a few cc. of tap water. A fresh solution of hypochlorite was made up and divided into two lots. To one lot was added the spore suspension. At intervals of 5, 10, and 15 minutes, 1 cc. lots were withdrawn with a pipette and discharged on to agar plates. Five plates were treated with 1 cc. of spore suspension at each time interval. Five plates were treated with 1 cc. of the original hypochlorite solution as control. The plates were swirled to distribute the liquid evenly over the surface and incubated at 25°C. After seven days, the result shown in Plate 8, Fig. 2, was obtained. Large numbers of spores were unharmed by immersion for as long as fifteen minutes, all plates being covered with colonies. It was apparent from inspection of the plates that the numbers of colonies fell off with increasing time of immersion, but no attempt was made to count the colonies.

A final experiment was then set up using a suspension of spores of approximately the same concentration, soaked in hypochlorite solution as before. One cc. lots were pipetted on to agar plates at 0, ½, 1, 1½, 2, 2½, 3, 3½, and 4 hour intervals. Two plates were treated with spore suspension and two with control hypochlorite solution at each time interval. After seven days' incubation, all control plates were sterile. Of the plates treated with spore suspension, those at 0 hour were covered with colonies, those at ½ hour bore three colonies, and one colony developed at 2 hours. The rest were sterile.

### 3. Discussion.

As shown by the two final experiments, the resistance of *Chaetomium* spores to hypochlorite is relative rather than absolute. The greater number of spores is killed by immersion in hypochlorite for half an hour, although an occasional very resistant one may apparently survive immersion for two hours. When, however, it is considered that the spores of all organisms settling on the plates during random contamination are killed by the small amount of chlorine gas diffusing from a few small drops of solution, the ability of *Chaetomium* spores to resist the actual solution for long periods is astonishing.

Examination of the species that fruited on the plates during these experiments revealed the following: (the classification of Chivers, 1915, is used) *C. globosum* Kunze, *C. spirale* Zopf, *C. cochliodes* Palliser, *C. funicolum* Cooke, *C. trilaterale* Chivers, *C. elatum* Kunze and Schmidt, *C. sphaerale* Chivers, and *C. bostrychodes* Zopf, as well as others which did not fruit under the conditions of the experiments. Of these *C. globosum* and *C. cochliodes* predominated. It is apparent that this peculiar insensitivity to chlorine is not confined to one or two species, but is characteristic of the genus. A search of the literature has revealed no previous reference to this phenomenon, although it is notable that the only contamination reported by Knudson (1933) while using hypochlorite was a *Chaetomium* sp. Its discovery presents interesting possibilities. Species of *Chaetomium* are commonly used in mildew-proofing tests of textiles (Thom *et al.*, 1934; Greathouse and Ames, 1945). They have also recently come into prominence as potential sources of antibacterial substances (Waksman and Bugie,

1944; Geiger *et al.*, 1944). The ability to collect species of *Chaetomium* in pure culture at any time by this simple technique should be useful in these studies. Particularly in the production of antibacterial substances it might be possible to save much time and labour by eliminating the necessity for painstaking sanitation. By flushing the apparatus with chlorine after setting up, it should be possible to eliminate contaminations without harming the *Chaetomium* being studied. Similarly the decontamination of stock cultures of *Chaetomium* may be accomplished by adding a little hypochlorite solution. The technique may also be used in the search for new species of the genus. A study of the literature indicates that species of *Chaetomium* are by no means uncommon, but that they seem to require rather special conditions, such as growth in dung of various kinds, before they become apparent in the fruiting stages. The exposure of plates in such localities as stable yards might reveal a number of new species.

It is thought that, apart from the question of isolating *Chaetomium* spp., the hypochlorite technique of surface sterilization is not sufficiently used by plant pathologists, despite its having been available for many years. It is an extremely effective sterilizing agent against organisms other than *Chaetomium* and may be substituted for the orthodox mercuric chloride in most instances. Plating the tissue direct from the hypochlorite solution without washing not only eliminates the need for preparing sterile washing water, but results in a great saving of time, which is a boon when making large numbers of routine isolations. But the most significant advantage of the technique lies in the automatic elimination of troublesome aerial contaminations, with the exception, of course, of an occasional colony of *Chaetomium*. Orthodox methods designed to eliminate or reduce these contaminations are no longer necessary, and plating may be done anywhere, even in the field.

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# A Note on Sterol Production by *Aspergillus flavus-oryzae* with Special Reference to its Anti-rachitic Potency.

*By W. J. Ellis, A.S.T.C.**

*Summary.*

The total sterol content of wheat bran mash is doubled by growing on it a mould of the group *Aspergillus flavus-oryzae*. The spectral absorption curves of alcoholic extracts of the mouldy bran resemble those observed for ergosterol, and the anti-rachitic potency for rats observed after drying the bran in the sun is equivalent to approximately 1,800 I.U. vitamin D per 100 g. of the water-extracted dry mouldy bran.

## 1. Introduction.

Sterol production by moulds has been investigated by Pruess *et al.* (1931, 1932), Yokoyama and Takata (1936) and Ramaswamy *et al.* (1943). Pruess reported that mould mycelium developed anti-rachitic activity on irradiation, but he did not estimate the activity quantitatively. In the present paper these findings are confirmed and the anti-rachitic potency developed on exposure of the mycelium to the sun is estimated.

The investigations were carried out in conjunction with the work of Lennox and Maxwell (1943), which was primarily concerned with protease production by a mould of the species *Aspergillus flavus-oryzae* when grown on wheat bran.

## 2. Methods.

(i) *Cultivation of the Mould.*—Since full details of cultivation will be published by Maxwell later, only a brief outline of the method will be included here. Wheat bran is moistened with water equal to 80 per cent. of the weight of the bran and spread on perforated trays in a layer approximately 1 inch thick. The trays are sterilized with steam in a cabinet. When cool, the steamed bran is inoculated by spraying with an aqueous suspension of mould spores, and growth is allowed to proceed for two to five days, during which time temperature and humidity are controlled. The bran culture is removed from the trays and extracted with water to remove the protease. Before measuring anti-rachitic potency, the residue is spread out on trays in thin layers and dried in the sun.

(ii) *Chemical Estimation of the Sterols.*—The digitonin method, described by Ramaswamy *et al.* (1943), was followed for the determination of unsaponifiable matter and total sterols in the original bran and in the mouldy bran residue. The alcoholic solution containing the sterols was submitted to spectrographic examination, and the spectral absorption curves drawn.

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(iii) *Biological Assay of Anti-rachitic Potency.*—The material used for assay was prepared by growing the mould as described above for four days, then extracting with water, drying the residue in the sun for seventeen hours, and grinding in a Wiley mill to 10 mesh. The rats used in the assay were of Wistar strain and were bred from a colony housed in this laboratory. When approximately 21 days old, at which stage they weighed between 40 g. and 60 g., they were kept for 21 days on the Steenbock rachitogenic diet No. 2965 (Steenbock and Black, 1925).

The 44 rats employed were divided equally into four groups. Two groups received the mouldy bran preparation, and two groups were given known dosages of vitamin D. A preliminary experiment was carried out to ascertain the maximum weight of mouldy bran consumed by one rat per day, when incorporated in the rachitogenic diet.

After a curative period, often 10 days, the rats were killed with coal gas. The proximal ends of the tibiae were X-rayed, and the negatives were compared with a series obtained from groups of rats which had been fed known amounts of vitamin D.

### 3. Results.

(i) *Chemical Analysis.*—The total ether-soluble matter, the unsaponifiable matter, and the total sterol content of bran and of the mouldy bran before and after water extraction are reported in Table 1.

TABLE 1.—STEROLS IN BRAN AND MOULDY BRAN.

| Material. | Ether-soluble Matter. | Unsaponifiable Matter. | Total Sterols. |
|---|---|---|---|
| | % | % | % |
| Steamed bran, sun-dried for 17 hr. .. | .. | 0·35 | 0·074 |
| Mouldy bran, sun-dried for 14 hr. .. | .. | 0·52 | 0·144 |
| Mouldy bran, water-extracted and sun-dried for 14 hr. .. .. .. .. | 3·37 | 0·84 | 0·202 |

It will be seen that the total sterol content of the mouldy bran was double that of the bran itself, also that extraction of the water-soluble constituents of the mouldy bran produced a significant increase in the sterol concentration.

(ii) *Spectrographic Analysis.*—The curves in Fig. 1 relate the wavelength to the extinction of a 1 cm. layer of a 1 : 100 dilution of the original alcoholic solution. The extinction is represented by the symbol $E^{1:100}_{1\ cm.}$ (Morton, 1942). Since the alcoholic extracts were not purified, accurate absorption curves were not obtained. There is strong evidence for the presence of ergosterol in sample No. 1 since the four

maxima coincide in position and almost in height. In sample No. 2 there seems to be a concentration of some substance absorbing strongly at 2670° A. Although the bran extract was rich in total sterols it did not exhibit any absorption bands.

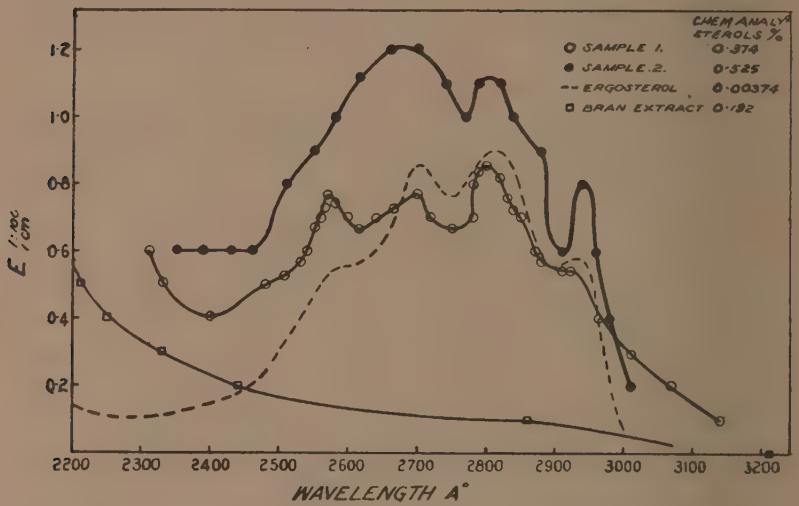


Fig. 1.—Absorption curves of alcoholic extracts of bran and mouldy bran.

(iii) *Biological Assay of Anti-rachitic Potency.*—Table 2 gives the figures obtained by the X-ray method for degrees of healing by mouldy bran in four groups of rats. The known dosages of vitamin D were administered in the form of standardized oil.

Table 2.—Anti-rachitic Activity of Mouldy Bran.

| Total Dose per Rat during Ten Days' Feeding. | Average Degree of Healing. |
|---|---|
| 1·5 g. mouldy bran .. .. .. .. | 6·45 |
| 0·5 g. mouldy bran .. .. .. .. | 5·00 |
| 6·0 international units vitamin D .. .. | 4·45 |
| 12·0 international units vitamin D .. .. | 5·55 |

It is apparent from these figures that water-extracted mouldy bran after exposure to the sun contained about 9 I.U. vitamin D per 0·5 g. or 1,800 I.U. per 100 g.

## 4. Discussion.

The vitamin D content of mouldy bran reported in this paper compares more than favourably with values reported for most foodstuffs, but it falls short of those reported for fish liver oils. However, the vitamin is extractable with lipoid solvents or oil, and the material might, therefore, serve as a source for the production of more potent preparations.

Preliminary experiments have shown that the mouldy bran residues have anti-rachitic value for chicks. But whether this signifies that they contain vitamin $D_3$ in addition to vitamin $D_2$ or whether the slight chick anti-rachitic of vitamin $D_2$ was sufficient to produce the observed effect, is not known. Since the provitamin $D_2$ ergosterol has been frequently detected in fungi, the latter seems to be the more likely explanation.

## 5. Acknowledgments.

The author is indebted to the Director of the Commonwealth Serum Laboratories for granting facilities for estimating anti-rachitic activity by the X-ray method, and to Dr. W. W. Hurst and Mr. A. G. Mathews for much assistance with this work. Thanks are also extended to Mr. R. J. Goldacre of the Physical Chemistry Section of this Division for determining the ultra-violet absorption curves of the extracts submitted, and to Miss M. L. Phillips for technical assistance.

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# The Etiology of Take-all Disease of Wheat.

## 1. A Survey of a Take-all Affected Field at Canberra, A.C.T.

*By N. H. White, M.Sc.**

*Summary.*

As part of a detailed survey of a take-all affected field at Canberra, some preliminary observations are recorded. The survey began the first year of cropping to wheat after conversion from a savannah-woodland natural pasture, and extended over a period of four years.

Take-all affected plants occurred singly or in small groups throughout the field, but conspicuously in large irregularly-shaped well-defined areas.

Soil from these areas and adjacent healthy areas did not show any significant difference in physical condition or in organic carbon and nitrogen content and in reaction.

Plants sampled at random from both areas and examined in the laboratory revealed that 64 per cent. from the take-all areas and 7 per cent. from healthy areas developed perithecia of *Ophiobolus graminis* on the bases of their culms, whereas, platings from both lots of plants yielded other fungi. From both lots of plants the species of fungi and the frequency of their occurrence were the same. They included *Fusarium culmorum* and *Helminthosporium sativum*.

During the first two seasons, when cropped to wheat, the take-all patches were in well-defined areas, but in the succeeding years, the diseased plants were widely distributed over the whole area. It was found that the location of the take-all areas shifted from season to season, although there was a tendency for them to be associated in succeeding years.

Seedling-blight symptoms in the field were associated with the presence of *Ophiobolus graminis*. The distribution of plants showing seedling-blight symptoms was of a fundamental type of clustering, suggesting the operation of a locality factor. It is considered that this locality factor is foci of inoculum of *O. graminis*.

Evidence was produced which indicated that local differences in soil condition other than those investigated, and the location of foci of inoculum of *O. graminis* may be the factors determining the position of take-all patches in the field.

### 1. Introduction.

The literature concerning the etiology of take-all disease of wheat has been comprehensively reviewed recently by Garrett (1942). With the exception of the investigations made by Russell (1934), most of our knowledge has been derived from separate contributions on certain aspects only, made in different localities. The opportunity to make a comprehensive study of the etiology of take-all in a particular locality came when a field of wheat, badly affected with take-all, some eight miles from the Division of Plant Industry's Laboratory at Canberra, was made available for these studies. The detailed investigation of the disease during a period of four years was made on a two-acre block of this field (shown in Plate 14) and the results are reported in this series of papers.

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## 2. History of the Field.

Before being sown to wheat for the first time in 1939, the area was typical savannah-woodland, with a natural pasture of the *Stipa-Danthonia* association under a canopy of *Eucalyptus melliodora* and *E. Blakelyi.* The composition of the pasture, as estimated by the pasture cover surrounding the field was as follows:—*Stipa variabilis, Danthonia pallida, Medicago minima, Erodium cicutarium, Vulpia bromoides, Hordeum leporinum, Bromus hordeaceus, Hypochaeris radicata, Sysymbrium orientale, Salvia Verbenaca, Bromus madritensis, Trifolium glomeratum, Capsella bursa pastoris, Plantago varia, Medicago procumbens, Gnaphalium* sp., *Lithospermum* sp.

In autumn, 1938 the trees were "grubbed" out and the stumps burned on this area. In spring, 1938, the whole area was cultivated and allowed to fallow until March, 1939, when it was again cultivated. The field was sown to "Bencubbin" wheat in April of that year. When the field was inspected in December, 1939, the wheat was showing evidence of a severe attack of take-all. It was at this time that a part of the field was made available to the Council for the study reported here.

## 3. Plan of Investigations.

The field was surveyed for differences which might contribute to the development of the symptoms of disease. This involved an examination of the soil and plants from diseased and apparently healthy parts of the field. The positions of the take-all patches were mapped from location points so that future references could be made to the position of the original patches. Work was then planned to obtain information on the following aspects of the disease:—

(i) Whether the position and size of the diseased patches varied season to season.

(ii) To observe the first appearance of the disease in the field and relate the location of these diseased plants to the subsequent development of take-all patches.

(iii) To examine the root system of plants during crop development and ascertain the microfloral content of these tissues during that period.

(iv) To analyse the factors concerned in the development of symptoms from two aspects: (*a*) the pathogenicity of organisms found associated with diseased root tissue when grown in soil, taken from the affected field, under controlled conditions; (*b*) relating the conditions of the root system to the shoot system in diseased and healthy plants taken from the field.

(v) To study the effect of agronomic practices and soil nutrients on the incidence and severity of the disease.

(vi) To attempt to discover the manner in which the pathogen is carried over from one season to another.

## 4. Examination of the Soil.

The soil of this field belongs to the soil group of red-brown earths (Prescott, 1931). A soil profile in the two-acre block was examined by Mr. J. K. Taylor of the Division of Soils, and was described as

follows:—"0–4 inches greyish-brown sandy loam, 4–12 inches light-brown sand to sandy loam, 12–24 inches yellowish-brown sandy clay loam, 24 inches becoming heavier to reddish clay loam with gravel."

Soil profiles were determined by sampling with the post-hole auger, at positions indicated in Fig. 1. Because characteristics of the soil profiles were the same in both take-all and healthy areas, and conformed to the description cited above, samples of the top 6 inches only of soil were obtained for analysis.

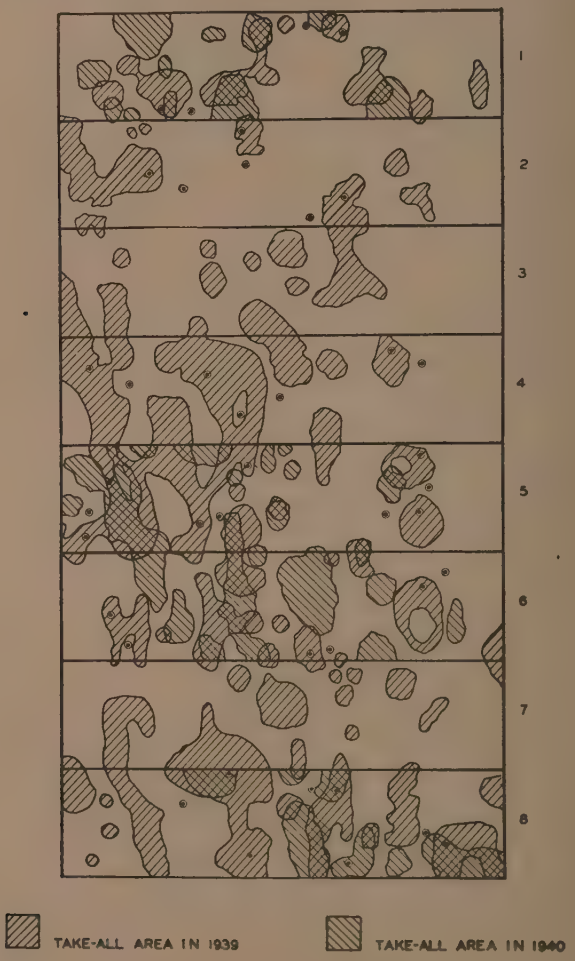


Fig. 1.—Location of take-all patches during two seasons in the two-acre survey area.

Twenty samplings were made at locations on take-all areas, and twenty on healthy areas (Fig. 1).

The physical nature of the top 6 inches of soil taken from the 40 locations indicated in Fig. 1 was ascertained by mechanical analysis. The Bouyoucos hydrometer method (Bouyoucos, 1930) was used for these determinations, and the results are shown in the mechanical analysis distribution triangle in Fig. 2.

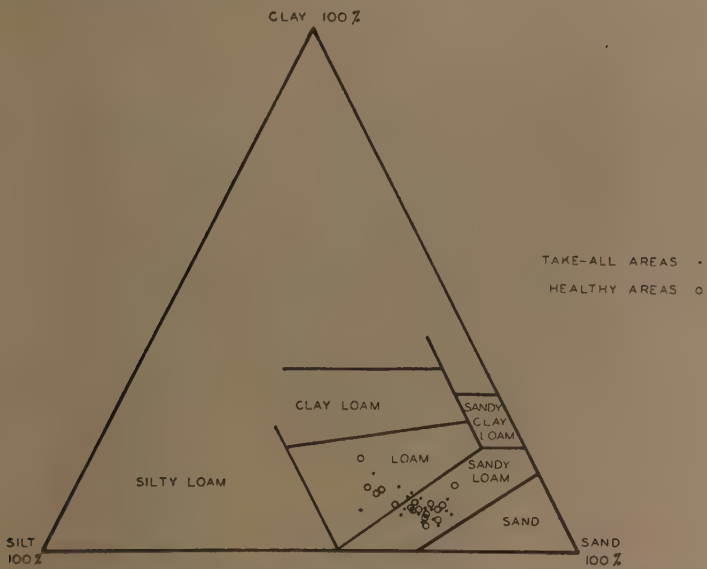


Fig. 2.—Mechanical analysis distribution triangle for the top six inches of soil.

From this it will be seen that the physical features of the top soils in both take-all and healthy areas show no significant differences.

Because high soil nitrogen and soil organic matter, as well as soil reaction, have been related to take-all disease by past workers, determinations for total nitrogen, organic carbon, and pH reactions were made on these samples. The pH was determined electrometrically with the glass electrode. The organic carbon was determined by Walkley's modification of the dichromate method (Walkley, 1935). The total nitrogen estimations were made according to the method of Prescott and Piper, Winkler's modification (Prescott and Piper, 1928). Although variations occurred in the organic carbon and nitrogen content for both lots of soil samples, there was no significant difference between soil from take-all areas and healthy areas. A summary of the results of these analysis is shown in Table 1.

Table 1.—Summary of Chemical Analysis on Soil Samples 0–6 Inches.

| | Take-all Area. | | | Healthy Area. | | |
|---|---|---|---|---|---|---|
| | pH. | Organic Carbon. | Total Nitrogen. | pH. | Organic Carbon. | Total Nitrogen. |
| | | % | % | | % | % |
| Mean .. .. .. | 6·7 | 1·127 | ·0722 | 6·7 | 1·000 | ·0665 |
| S.E. of Mean .. | ·270 | ·098 | ·0066 | ·270 | ·098 | ·0066 |

For these physical and chemical soil properties it is evident that major differences did not occur between the top soils from take-all and healthy areas. This does not imply that organic matter, nitrogen content, and reaction of the soil may not influence the development of take-all. Garrett (1942) concluded that these soil factors are important in the incidence and severity of the disease. Again, although the soil texture and soil profile in diseased and disease-free areas did not differ, it is well known that the disease is favoured by light-textured soils.

The analyses made from this field indicate that if the soil conditions here are the determining factors in the position of take-all patches, these conditions are likely to be less obvious ones, perhaps differences in available plant nutrients (Angell, 1943, 1945), including the trace elements (Millikan, 1941).

## 5. Examination of the Plants.

In December, 1939, plants were sampled at random from both take-all areas and apparently healthy areas. All plants from take-all areas showed the typical blackening of the stem bases and the crown roots, with plates of dark-coloured mycelium covering the culms. Some of the plants taken from the apparently disease-free areas also showed these symptoms, but most of them had a healthy root system.

A mycological examination was undertaken to discover differences in the fungal population of the subterranean parts of plants from take-all and from healthy areas.

Roots and portions of the crowns of the plants from both areas were plated on potato dextrose-agar, after surface sterilization with mecuric chloride. The remaining portions of the culms of these plants were placed in moist sand in separate tubes and then after six weeks were examined for fruiting bodies of fungi.

The platings gave a large number of species of fungi shown in Table 2, including *Helminthosporium sativum, Fusarium culmorum,* and *Fusarium* spp., but none gave *Ophiobolus graminis* the fungus usually associated with take-all disease.

TABLE 2.—THE RESULTS OF MYCOLOGICAL EXAMINATIONS OF PLANTS FROM DISEASED AND HEALTHY AREAS.

| Area Sampled. | Number of Plants Examined. | Percentage of Plants Plated Yielding— | | | Percentage of Plants with Perithecia of *O. graminis*. |
|---|---|---|---|---|---|
| | | *Fusarium*. | *Helmintho-sporium*. | Other Fungi. | |
| Diseased .. | 150 | 59 | 13 | 83 | 64 |
| Healthy .. | 183 | 55 | 11 | 76 | 7 |

The dominant organisms isolated in these plates were the same and of the same frequency in plated material from both take-all and healthy areas. However, when the remaining portions of the culms of these plants were examined, after being kept in moist sand for six weeks, it was found that mature perithecia of *O. graminis* were embedded in the sheathing bases of 64 per cent. of the plants sampled from take-all areas, but only on 7 per cent. of the plants sampled from the apparently healthy areas, as shown in Table 2.

Experience has shown that is is difficult to isolate *O. graminis* by plating diseased material from mature plants, even after the plants had been thoroughly infected with this fungus (Sadasivan, 1939). The development of perithecia of *O. graminis* on the culms seems to be the most satisfactory way of estimating the distribution of this fungus in a crop that is sampled at maturity.

When perithecial development of this fungus on the two lots of material is considered in relation to the results of plating the material, as in Table 2, it will be seen that the only significant difference is in the high yield of *O. graminis* on plants sampled from take-all areas as compared wth the yields of this fungus on plants taken from the healthy areas. The development of perithecia on 7 per cent. of the plants taken from healthy areas, is explained by the fact that take-all plants occurred singly or in small groups amongst the normal stands of wheat in this field, and that in sampling, such plants were not eliminated.

## 6. Distribution and Association of Take-all Patches in Successive Seasons.

In order to determine whether the position and size of take-all patches varied from year to year, the take-all areas in the two-acre block were mapped to scale in December, 1939, and those portions of it carrying wheat again in November, 1940. This is shown in Fig. 1. In 1940 the two acres were divided into eight blocks, each measuring 200 ft. by 50 ft. Wheat was sown only in plots 1, 5, 6, and 8 in that year, therefore the position of the take-all areas could be mapped for these plots in 1940 only. It will be seen that the take-all areas vary considerably in shape and size and that the position of the areas did not remain the same for each season, although there was a tendency for the areas to be associated in successive seasons. The amount of over-lapping in the two seasons may be seen in the cross-hatched areas in Fig. 1.

In 1941 wheat was sown on plots 5 and 6 only, and, therefore, the extent of the take-all areas could be observed only on these plots. However, the boundaries of the diseased areas were so poorly defined this year that it was impossible to map them.

In 1942, the whole of the two acres, including plots 5 and 6, was sown to wheat, but no well-defined take-all areas occurred, although the disease was present in most of the plots.

In plots 5 and 6, sown continuously to wheat for three successive seasons, take-all plants occupied the whole of the area, and there were only a few plants not showing the diseased condition.

In both the 1939 and 1940 seasons, plants showing take-all symptoms occurred either singly or in small groups throughout the crop as well as in the well-defined areas mapped in Fig. 1.

The occurrence in this field of single or small groups of take-all affected plants scattered throughout the crop as well as in the defined areas, agrees with the experience of past workers (Garrett, 1942). However, there appears to be no published record of any attempt to relate the position of take-all patches in succeeding years for a particular field. This would only be possible if the patches were well-defined, as they are during the first few years after the appearance of the disease. Russell (1934) kept the same field under observation for a number of years, but did not report any details on the relative position of the diseased patches during succeeding seasons, except that they appeared to change in size and shape.

The shifting of the position of well-defined areas of take-all in the first two years, and the final covering of the whole area with the disease in subequent years, suggest the operation of either one or both of two factors, namely: (i) local differences in soil condition, such as soil fertility as mentioned earlier in Section 4, which became less accentuated with continuous cropping; and (ii) foci of inoculum, which gradually became dispersed over the whole area in subsequent years.

### 7. Distribution of Plants Showing Disease Symptoms at the Seedling Stage.

In June, 1940, a careful examination of the field revealed the presence of plants with seedling-blight symptoms. These symptoms were identical with those obtained by experimental inoculation in potted soils. They were characterized by the following:—(i) stunted growth and no jointing; (ii) yellowing and death of first and second leaves; (iii) rolling and yellowing of the youngest leaves. Normal plants as well as a large number of plants showing symptoms were examined. All plants showing symptoms were found to have varying numbers of their seminal roots with lesions and the typical runner hyphae of *O. graminis*. With some exceptions the symptomless plants had seminal roots without lesions. All lesions gave colonies of *O. graminis* on plating after surface sterilization. Other than *O. graminis*, no fungi, with few exceptions, were isolated from these lesions. It would seem that *O. graminis* was the cause of seedling-blight in this field. It was considered that if the disposition in the

field of plants with seedling-blight symptoms was known then the distribution of foci of inoculum of *O. graminis* would be known. To determine whether the plants with disease symptoms were distributed at random or clustered in localized areas, an area 60 ft. by 50 ft. on plot 5 was mapped, giving the locations of every healthy and diseased seedling plant in July. A small section of the mapped area is shown in Fig. 3.

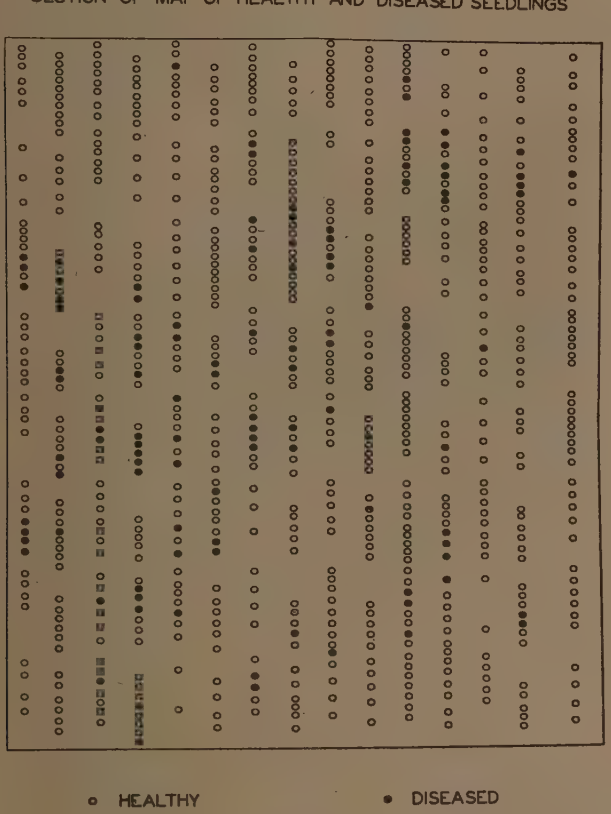


FIG. 3.—Section of map of healthy and diseased seedlings.

The data were then analysed according to the methods used by Cochran (1936).

(i) *Test for Clustering of Diseased Plants in Areas.*

The mapped area was subdivided by rulings into small plots approximately square and three rows wide, each plot containing twelve plants. The observed frequencies of plots with 0, 1, 2, 3, &c., diseased plants were tabulated, and the expected frequencies for the binomial distribution with the same proportion of diseased plants and same number of plots were calculated. The data are given in Table 3.

The $\chi^2$ test for the agreement between observed and expected frequencies gives $\chi^2 = 161{\cdot}48$ for two degress of freedom ($P < {\cdot}001$), which indicates that diseased plants occur together more frequently than one would expect by chance.

TABLE 3.

| Diseased Plants. | Frequency (Observed). | Frequency (Expected). | Difference. | $\frac{(\text{Difference})^2}{\text{Frequency (Expected).}}$ |
|---|---|---|---|---|
| 0 .. .. | 819 | 730·15 | 88·85 | 10·81 |
| 1 .. .. | 212 | 328·76 | 116·76 | 41·47 |
| 2 .. .. | 64 | 67·84 | 3·84 | ·22 |
| 3 .. .. | 20 | 8·47 | | |
| 4 .. .. | 41 14 | 9·25 ·78 | 31·75 | 108·89 |
| 5 .. .. | 5 | | | |
| 6 .. .. | 2 | | | |
| | 1,136 | 1,136 | .. | 161·48 |

(ii) *Test for Occurrence of Diseased Plants in Rows.*

Following Cochran (1936), a test was made of the signficance of departure of the frequency of runs of diseased plants from the expected frequency which follows a geometrical progression law. The observed and expected frequency distributions are shown in Table 4.

TABLE 4.—OBSERVED AND EXPECTED FREQUENCY DISTRIBUTIONS OF DISEASED PLANTS.

| Length of Run. | Frequency (Observed). | Frequency (Expected). |
|---|---|---|
| 1 .. .. .. .. | 352 | 399·03 |
| 2 .. .. .. .. | 51 | 14·43 |
| 3 .. .. .. .. | 6 | ·52 |
| 4 .. .. .. .. | 4 | ·02 |
| 5 .. .. .. .. | 1 | ·00 |

There was a greater tendency for two or more diseased plants to occur together than would be expected by chance.

(iii) *Test for Mode of Clustering of Diseased Plants.*

Having found clustering in both rows and in small units of area, it was necessary to determine whether the clustering in areas could be attributed to the disease occurring in sequences along rows, each row being independent of neighbouring rows, or whether the sequences in rows occurred because the rows cut across localized areas of disease.

A test to discriminate between these alternatives was made by finding if the sequences were independently distributed in adjacent rows. The plants in pairs of adjacent rows were divided into sets, each set

containing three plants in each row. Any omitted plants between consecutive sets were discarded because there was no plant opposite or nearly opposite in the adjacent row. The record of the number of diseased plants out of three in the two rows of each set were tabulated in the form of a contingency table. The frequencies of occurrence of 1, 2, and 3 diseased plants have been grouped for purposes of calculating $\chi^2$. The data are given in Table 5.

Table 5.

| | | Diseased Plants in First Row of Set. | | |
|---|---|---|---|---|
| | | 0. | 1, 2, 3. | Total. |
| Diseased Plants in Adjacent Row. | 0 .. .. | 1,138 | 94 | 1,232 |
| | 1, 2, 3 .. | 116 | 35 | 151 |
| | Total .. | 1,254 | 129 | 1,383 |

This gives $\chi^2 = 38{\cdot}48$ for 1 degree of freedom which is highly significant. Hence the evidence indicates that, fundamentally, there is a locality factor favouring the occurrence of diseased plants and that the clustering along rows is produced by the rows cutting across such areas.

The occurrence of seedling-blight in the field at an early stage in take-all disease was first recognized by McAlpine (1904), and since then other workers observed the same stage (Russell, 1934; Garrett, 1942). In all the records of this stage of the disease, it is stated that the plants with seedling-blight occurred in more or less circular patches and were associated with the white-head condition or final stage because it was at that time that the fields were examined for the disease.

Exact observations on the distribution of seedling-blight are not reported in the literature. In the above field, the plants with seedling-blight were observed at the seedling stage of crop development. It was found from the analysis of the data on the position of these diseased seedlings that they occurred in patches, which may be taken to indicate the operation of a locality factor.

The occurrence of lesioned roots yielding *O. graminis* almost entirely, suggests that this locality factor is foci of inoculum of *O. graminis*.

## 8. Acknowledgments.

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# The Etiology of Take-all Disease of Wheat.

## 2. Progressive Necrosis and Microfloral Succession in Root and Crown Tissue of Wheat.

*By N. H. White, M.Sc.**

*Summary.*

1. During the development of a wheat crop naturally affected by take-all, there was an increase in the amount of diseased tissue, first in the seminal roots, then in the crown roots, and finally in the crowns of the wheat plant.

2. There was an increase in the number of plants with lesions as the crop developed, and at the ripe stage all plants had root lesions.

3. The distinction between the condition of the roots of white-eared and normal-eared plants in this field was one of degree only.

4. The lesions were caused by a primary invading organism, *Ophiobolus graminis*.

5. In the tissue lesions of the roots and crowns, there is a microfloral succession which results in the disappearance of *O. graminis*.

6. Organisms isolated from the basal tissues of wheat plants showing take-all symptoms may not have a true causal relation to the disease, but instead represent the dominants of a climax fungal community found in senescent root tissue.

### 1. Introduction.

Observations made in a take-all affected field at Canberra during the 1940 season indicated that the basal parts of wheat plants became progressively diseased as the season advanced, and although most of the plants had diseased tissue at maturity, only a proportion of them became white-eared plants. In 1941, a more accurate study was made of the progressive necrosis of root and crown tissue, and an attempt was made to relate the amount of tissue destruction during crop development to the final condition of the plant. At the same time, the microfloral content of diseased and healthy basal parts of the wheat plant was determined at different stages of crop development to obtain some idea of the primary cause of the progressive destruction of the tissues.

### 2. Method.

When the crop was sown late in April, 1941, small quadrats were placed at random positions in the field. Each quadrat contained about 60 plants, and samplings were made of five quadrats at each of the following stages of crop development: seedling, jointing, pre-boot, in-boot, anthesis, dough, ripe and stubble; at each sampling, the plants in five quadrats were dug up and brought to the laboratory for examination. This material was used for obtaining data on progressive necrosis of the tissues and the microfloral content. At each sampling date, health ratings were made on plants in all the quadrats, including those that

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were to be sampled at that time. The health ratings were based primarily on the amount of dead leaf tissue, turgidity of the leaves, and height; the number of tillers was an associated factor. In the laboratory, the following data were obtained for each plant: number of tillers, height, number of healthy and diseased seminal roots, number of healthy and diseased crown roots, and diseased sub-crown internodes and crowns.

As far as possible platings of the tissues were made on the day of sampling, and between 80 and 200 pieces of tissue were plated. For isolation work pieces of diseased roots were taken at a uniform distance of 5–10 cm. from the crown. A few pieces were plated from each plant, but all diseased roots were not plated, since it was found from preliminary work that sampling every time from about the same distance from the crown obviated the necessity of plating every root lesion.

In making the isolations, a piece of root 2 cm. long was immersed in 50 per cent. ethyl alcohol for 20 seconds, and in 1 in 1,000 parts mercuric chloride solution for 1½ minutes, and then washed four times in sterile water. The surface-sterilized tissue was cut into six 3 mm. lengths and plated so that the cut ends were embedded in the potato-dextrose agar medium. The crowns were similarly treated, except that after surface sterilizing they were quartered and then plated. By adopting this procedure, only organisms actually inside the tissues were isolated. Platings of healthy roots and crowns were also made at each sampling. At each sampling the number of plates yielding each type of organism was recorded, and the fungal and bacterial types were maintained in sub-cultures for identification.

### 3. Results on Progressive Necrosis.

A summary of data obtained on the progressive necrosis of the root and crown tissue is shown in Table 1. With crop development there was an increase in the amount of diseased tissue first in the seminal

Table 1.—A Summary of Data on the Progressive Necrosis of the Roots and Crowns of Random Samples of Wheat Plants.

| Date of Sampling .. .. .. | 10th Sept., 1941. | 7th Oct., 1941. | 5th Nov., 1941. | 18th Dec., 1941. |
|---|---|---|---|---|
| Stage of Development .. .. .. | Late Tillering. | In-boot. | Anthesis. | Ripe. |
| Number of plants examined .. | 237 | 276 | 228 | 303 |
| Mean percentage of lesioned seminal roots per plant .. .. .. | 19·4 | 45·3 | 68·2 | 83·7 |
| Percentage of plants with lesioned seminal roots .. .. .. | 51·0 | 76·5 | 88·7 | 99·0 |
| Mean percentage of lesioned crown roots per plant .. .. .. | 7·6 | 30·8 | 45·1 | 56·8 |
| Percentage of plants with lesioned crown roots .. .. .. | 39·2 | 87·3 | 96·9 | 99·7 |
| Percentage of plants with lesioned roots .. .. .. .. | 57·9 | 82·5 | 97·8 | 100·0 |
| Percentage of plants with lesioned crowns .. .. .. .. | 0·0 | 10·1 | 21·0 | 41·2 |

roots, then in the crown roots, and finally the crowns. At the same time, there was an increase in the number of plants with lesions on these tissues, and at the ripe stage all plants had root lesions. However, only 17 per cent. of the plants sampled at anthesis were white-eared plants, i.e., showing the typical take-all condition. It appears that the distinction between the root condition of white-eared plants and plants with normal ears in these quadrats was one of degree only, that is, the plants whose root systems were most severely attacked succumbed and became white-eared plants. This fact is supported by data in Table 2

TABLE 2.—ESTIMATED DEGREE OF DAMAGE TO ROOTS AT EACH SAMPLING OF PLANTS THAT WERE SUBSEQUENTLY EITHER WHITE-EARED OR NORMAL.

| Sampling Date. | Normal Plants. | | White-eared Plants. | |
|---|---|---|---|---|
| | Crown Roots Lesioned. | Seminal Roots Lesioned. | Crown Roots Lesioned. | Seminal Roots Lesioned. |
| | % | % | % | % |
| 10th September, 1941 .. .. | 7·69 | 18·48 | 8·14 | 21·14 |
| 7th October, 1941 .. .. .. | 27·46 | 38·30 | 31·07 | 45·73 |
| 5th November, 1941 .. .. | 39·13 | 60·52 | 51·72 | 75·64 |
| 18th December, 1941 .. .. | 45·07 | 76·91 | 73·54 | 94·57 |

which give the estimated amount of damage to the roots at each sampling date of plants which subsequently became white-eared or appeared to be normal. These estimates were obtained by determining the distribution of health ratings of the white-eared and normal-eared plants at each sampling date, and the percentage of root tissue damaged for plants of each health rating in the sampled quadrats. It was assumed that plants with the same health rating would have the same degree of root damage, irrespective of whether their final condition was to be white-eared or normal. Only for the first date on which health ratings were made (1.7.41) is the difference in health of subsequently normal and white-eared plants not significant, as seen in Table 3.

TABLE 3.—COMPARATIVE HEALTH RATINGS* OF SUBSEQUENTLY NORMAL AND WHITE-EARED PLANTS.

| Date. | Normal-eared. | White-eared. | Significance of Difference. |
|---|---|---|---|
| 1st July, 1941 .. .. .. | 0·22 | 0·26 | n.s. |
| 10th September, 1941 .. .. | 0·35 | 0·95 | $p = <·001$ |
| 7th October, 1941 .. .. .. | 2·04 | 2·43 | $p = <·001$ |

* Scale of health ratings—0 = healthy to 4 = very poor.

### 4. Results on Microfloral Succession.

At the seedling stage, seminal roots were the only roots with lesions, and platings of these gave 100 per cent. yield of *Ophiobolus graminis*. There were some bacterial colonies, but no other fungi were isolated at this sampling. From the jointing stage onwards, platings of root lesions were made from crown roots only. Special attention was given to the relation of the percentage frequency of isolations of *O. graminis* to other fungi at each of the samplings. This relationship for platings of lesions, both on roots and crowns, is shown in Fig. 1.

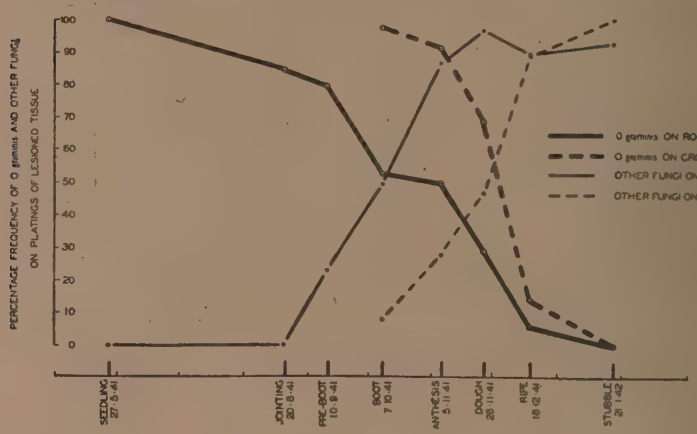


Fig. 1.

It will be seen that the percentage frequency of *O. graminis* isolations from plated root and crown lesions fell off as the season advanced until at the ripe stage the fungus was not isolated at all, whereas the percentage frequency of total other fungal isolations from these tissues increased to a maximum at the ripe and stubble stages. Although lesions were not found in the crowns until "in-boot" stage, the same relationship held for the frequency of *O. graminis* isolations to other fungi. Platings of tissue without lesions did not yield any fungi until the dough, ripe, and stubble stages, but *O. graminis* was never isolated from these tissues.

Although many species of fungi and bacteria were isolated from diseased tissue at different stages of development, some of them were found only in an odd plate at the different samplings. These were species of *Aspergillus, Mucor, Pythium, Cephalothecium,* and *Rhizopus nigricans,* as well as some bacteria. Twenty organisms were recognized as dominant types, and the frequencies of these are given in Table 4, where the rating used is similar to that suggested by Weaver and Clements for estimating the abundance of species in frequency quadrats in plant ecology. In this scale, the frequency ratings are 1–5 per cent. = Rare (R), 6–15 per cent. = Infrequent (I), 16–35 per cent. = Frequent (F), 36–80 per cent. = Abundant (A), and 80–100 per cent. = Very Abundant (V.A.).

TABLE 4.—FREQUENCIES OF THE DOMINANT COMPONENTS OF TOTAL OTHER FUNGI, AND BACTERIA IN CROWN ROOT LESIONS AT EACH SAMPLING.

| Organism. | Frequency Rating. | | | | | |
|---|---|---|---|---|---|---|
| | Pre-boot 10th Sept., 1941. | In-boot 7th Oct., 1941. | Anthesis 5th Nov., 1941. | Dough 26th Nov., 1941. | Ripe 18th Dec., 1941. | Stubble 21st Jan., 1942. |
| *Wojnowicia graminis* .. | R | R | I | R | I | I |
| *Helminthosporium sativum* .. | 0 | 0 | 0 | R | I | I |
| *Curvularia ramosa* .. | 0 | R | 0 | I | R | I |
| *Trichoderma lignorum* .. | 0 | R | 0 | R | I | R |
| *Trichoderma* sp. .. .. | R | R | I | 0 | R | R |
| *Alternaria* sp. .. .. | R | R | F | F | F | A |
| *Penicillium* sp. I. .. | 0 | R | 0 | R | I | I |
| *Penicillium* sp. II. .. | 0 | R | 0 | R | R | R |
| *Fusarium culmorum* .. | 0 | 0 | R | I | F | F |
| *Fusarium oxysporum* .. | 0 | 0 | 0 | R | 0 | I |
| *Fusarium* sp. I. .. .. | R | I | F | F | F | F |
| *Fusarium* sp. II. .. .. | 0 | R | I | I | R | F |
| *Fusarium* sp. III. .. | 0 | R | I | R | F | I |
| *Chaetomium* sp. .. .. | R | I | I | F | I | F |
| *Sordaria* sp. .. .. | R | R | I | F | R | I |
| Sterile mycelium (M5) .. | R | I | F | I | I | I |
| Bacteria type I. .. .. | I | F | F | F | F | F |
| Bacteria type II. .. .. | R | F | A | F | A | F |
| Bacteria type III. .. | I | I | F | F | I | A |

R = Rare. I = Infrequent. F = Frequent. A = Abundant. V.A. = Very Abundant.

It will be seen that these organisms in the diseased crown root tissue may be placed into one of three groups according to their frequency trends over the sampling period. These groups are:—

(i) increasing in frequency;

(ii) frequency remaining constant; and

(iii) fluctuating in frequency.

When compared, it will be noted that none decreased in frequency other than *O. graminis,* whereas a number of different organisms belonged to each of the three groups of frequency trends. As the season advanced, *Fusarium* spp. increased in abundance until at the stubble stage they were the dominant fungi isolated from both diseased and normal senescent tissue alike.

## 5. Discussion.

It has been shown that the seminal roots, crown roots, and crown tissues became progressively diseased as the crop developed. Initially lesions in each of these tissues all gave cultures of *O. graminis,* but later samplings yielded less of *O. graminis* cultures, and more of other fungi. Parallel platings of healthy tissues showed at first that no organisms were present, but when they became senescent at the dough and ripe stages, they were populated with fungi and bacteria similar to those found in already diseased tissue. These observations suggest that the tissues of the basal part of the developing wheat plant may be

invaded by *O. graminis* which permits the entry of the many saprophytes usually associated with senescent wheat root tissue, and these replace the primary invading fungus.

Much has been published on the antagonism of fungi and bacteria to *O. graminis* on culture media, Sandford and Broadfoot (1931), Henry (1932), Broadfoot (1933A and 1933B), Garrett (1934, 1936 and 1938), and Lal (1939), and the decline in the frequency of *O. graminis* isolations at each of the successive samplings may be explained on the basis of antagonism.

The results of this investigation give ample proof to the suggestion made by Garrett (1936) that the relation between *O. graminis* and other soil inhabitants should be considered from an ecological point of view, and that there seems to be a regular succession in diseased wheat roots. Considered ecologically, the initial cause of succession in wheat tissue is the presence of the pathogen *O. graminis* which invades the healthy tissues causing lesions. These lesions provide a non-living substrate suitable for the growth of saprophytes. *O. graminis* is then replaced by the saprophytes in these lesions. The presence of these organisms is apparently inimical to the growth of *O. graminis*; this may be due to competition for food by the more rapidly growing organisms, since *O. graminis* is a relatively slow growing fungus. From the first primary lesions, runner hyphae invade more healthy tissue causing fresh lesions, and again the replacement is repeated. The character of succession in each lesion will be determined by the climatic conditions of the rhizosphere, the chief factors would be aeration, temperature, moisture, and reaction.

Simmonds and Ledingham (1937) made a survey of the prevalence of fungi in or on wheat roots at various soil levels, samples being taken at seedling stage and early maturity, but no effort was made to take only parts bearing lesions; 27 genera of fungi were obtained and 50 per cent. of these were considered pathogenic. Millikan (1941) determined the relative abundance of fungi associated with wheat root lesions at jointing and ripe stages and found "that a complex succession of fungi was associated with the root rot lesions". The writer found that the components of the climax fungal community in tissue lesions were the same as those found in non-lesioned tissues of senescent plants; also that *Fusarium* spp., including *F. culmorum*, and *Helminthosporium sativum* were the dominants. Other workers have also found *Fusarium* spp. predominant in platings of mature root tissues.

Broadfoot (1934) examined a large number of mature wheat plants irrespective of disease symptoms and found that 92 per cent. yielded *F. culmorum*, *Fusarium* spp., and *H. sativum*, either alone or in combination with other fungi. Samuel and Greany (1937) made isolations from both diseased and healthy plants at harvest time and found *F. culmorum* and other species of *Fusarium* on most of both lots of plants. Subsequently, they began isolation work at flowering time in order to obtain some idea of the progressive invasion of the roots and crowns by fungi. They found the amount of *F. culmorum* increased as the season advanced. Also they observed that, although there was an appreciable amount of *F. culmorum* present on the roots, the crop gave excellent yields and no disease. Millikan (1941) reported that from root

lesions on mature wheat plants grown on red soil of the Wimmera, over 80 per cent. of the isolations consisted of either one of *F. culmorum, F. scirpi* var. *compactum,* and *F. moniliforme.* During the 1940 season, the writer obtained a high yield of *F. culmorum, Fusarium* spp., and *H. sativum* from plants sampled at the dough to ripe stage from a number of disease-free fields in the central wheat belt of New South Wales. In view of the above observations, it would seem that fungi isolated from the basal parts of mature wheat plants showing take-all symptoms may not necessarily have a causal relation to the disease, but instead represent a climax fungal community, the result of microfloral succession after the formation of lesions by a primary parasite. Experience has shown that it is difficult to isolate *O. graminis* from the basal parts of wheat plants when sampled at the mature stages. Garrett (1936) has stated that the succession revealed by the culture plate "explains the difficulty experienced by workers on cereal foot-rots in isolating the causal agent from a root that is not in the earliest stages of infection". In the study reported here, the writer was able to plate roots in the earliest stages of infection, and the organism isolated was *O. graminis.*

## 6. Acknowledgments.

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# Mineral Deficiency in Plants on the Soils of the Ninety-mile Desert in South Australia.

## 1. Preliminary Investigations on the Laffer Sand, near Keith.

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*Summary.*

1. An investigation into the mineral nutrition of plants grown on the poor soils of the Ninety-mile Desert has been undertaken. Results of experiments on the Laffer sand are described.

2. Cereals and pasture species grown without the addition of phosphate remained extremely dwarfed and developed marked symptoms of phosphorus deficiency. Addition of phosphate permitted vigorous growth, and maximum yields were obtained when superphosphate was applied at the rate of 2 cwt. per acre.

3. A deficiency of two trace elements was revealed, and responses to their application depended upon the species of plant concerned. The yield of oats was considerably increased by a dressing of zinc sulphate; lucerne showed a response to copper sulphate; and the yield and particularly the seed production of subterranean clover was improved by the addition of zinc sulphate and copper sulphate together.

4. Oats grown with liberal dressings of superphosphate alone continued to exhibit a marked discolouration suggestive of phosphorus deficiency. Zinc reduced the discolouration, but more completely at a low than at higher levels of phosphate. The symptoms were not alleviated by nitrogen, potassium, or copper.

5. Census studies showed that the increase in the grain yield of oats obtained with the first increment of phosphate was attributable mainly to an increase in the number of grains per head, while a second increment of phosphate increased the number of heads produced. The addition of zinc brought about an increase in the number of grains per head.

6. It is evident that the trace elements zinc and copper will play an important part in the development of the vast area of this and related soils in the Ninety-mile Desert in South Australia and Victoria.

### 1. Introduction.

A study of the mineral nutrition of plants grown on the heath sands of the Ninety-mile Desert in the upper South-east of South Australia was commenced in 1944. The occurrence of a trace element deficiency had been suspected there for some time from evidence obtained in a number of widely separated localities.

A soil survey of the Hundreds of Laffer and Willalooka in the Ninety-mile Desert was carried out in 1932 by the Council's Division of Soils at the request of the South Australian Department of Lands, and in the report of the findings (Taylor, 1933) the approximate boundary of the related mallee and heath soils was outlined. The greater part of the upper South-east of South Australia and a considerable portion of north-western Victoria, some 10,000 square miles

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in all, is comprised of poor soils of this type. On account of this, agricultural development in the past has been very limited, but more clearing and cultivation has been undertaken in recent years.

The natural vegetation of the Desert is characteristic. It consists, for the greater part, of a low scrub of various heath plants, broombush (*Melaleuca* spp.), oakbush, (*Casuarina* spp.), yacca (*Xanthorrhoea semiplana*) and stunted *Banksia* spp., with sometimes stunted mallee (*E. oleosa* and *E. angulosa* or *E. diversifolia*) or pink gum (*E. fasciculosa*). The soils are light-textured grey and white sands of varying depth over clay-loam and limestone.

The climate is suitable for the production of cereal crops and sown pastures. The average annual rainfall in the northern portions is 15 inches per annum. At the southern limits it reaches 20 inches or more per annum. The mean length of growing period is 6·0 months in the north and over 7·5 months in the south (Trumble, 1937).

The limited area under cultivation has been devoted mainly to cereal cropping. Reports and observations revealed that even where phosphatic fertilizers had been applied liberally the growth of sown pastures was poor and they failed to persist. Occasionally lucerne was grown with some measure of success. Evening primrose *Oenothera odorata*) seemed generally to be preferred to any other type of pasture plant.

Experiments were commenced in 1944 to determine primarily the response of a number of pasture species to the supply of various trace elements. One experiment also was devoted to a study of the effect of these trace elements on the growth of the cereal oats. These plants have proved particularly useful as indicator plants as they develop more rapidly than the pasture species and exhibit deficiency symptoms which can be more easily recognized. This latter experiment has been completed and the results are recorded here. The pasture experiments are to be observed and the responses assessed quantitatively for several years; however, some results obtained during the first year will be discussed briefly in this paper.

## 2. Experimental Site.

The site selected for the experiments was 8 miles south-west of Keith, on the normal phase of the Laffer sand (Taylor, 1933) immediately adjacent to an area of the woodland phase of the same soil type. The soil here consists of a layer of grey sand about 4 inches deep which merges into about 9 inches of white sand and then to greyish-yellow sandy clay loam, with limestone at a depth of about 19 inches. The natural vegetation over this area was low scrub, seldom more than 3 feet high, of an association of various heath plants, stunted *Banksia* spp., oakbush (*Casuarina Muelleriana*), yacca (*Xanthorrhoea semiplana*), broombush (*Melaleuca fasciculiflora*) and occasionally stunted mallee (*E. oleosa* and *E. angulosa*) (Plate 9, Fig. 1.).

In 1940 the particular area had been ploughed ineffectively to a shallow depth without prior logging or preliminary burning of the scrub. It was then abandoned. The scrub had regenerated to almost its original height and density by January, 1944, when it was ploughed

in the dry state to a depth of 9 to 10 inches with a heavy disc (Majestic) plough. The natural vegetation was neither logged nor burned but was completely turned under by the deep ploughing. In April the area was harrowed, and when the larger stumps had been removed, cross-ploughed to a depth of 4 to 5 inches, this time with a twin-disc plough. The land was then harrowed, cleared of the worst stumps and cultipacked immediately prior to being sown on May 26, 1944. A thoroughly firm seed-bed was obtained by the cultipacking operation, and the soil surface was left in a suitable condition for the passage of sowing and harvesting machinery (Plate 9, Fig. 2).

Rainfall for 1944 was below average. Keith township, 8 miles to the north-east, was the nearest official station at which rainfall records had been kept for any length of time, and the average rainfall there over a period of 36 years was 17·84 inches. In 1944 only 12·36 inches were recorded for the year at Keith, and 14·28 inches were recorded at the experimental site. The average length of the growing period in the district is considered to be rather less than 7·5 months (Trumble, 1937). The average rainfall over the May-November growing period at Keith is 13·32 inches; the fall over this period in 1944 was nearly 5 inches short. Ten inches of rain fell at the experimental site during the growing period, and August, with only 22 points of rain, was exceptionally dry.

Many frosts occurred during July, August, and September, some of which were particularly severe. Crops and pastures in the experimental area suffered some damage but recovered well after rains which fell in September and October.

### 3. Procedure.

The object of the cereal experiment was to determine primarily the effects of zinc and copper on the growth of oats sown as indicator plants on the heath sand described above. Owing to the frequent occurrence of interactions in experiments of this nature, it has been found necessary to study at the same time the effects also of phosphorus, nitrogen, and potassium. Factorial design was therefore employed.

The various fertilizers used and their rates of application per acre, were as follows:—

Superphosphate—nil, 1 cwt., 2 cwt., 4 cwt.
Blood manure—nil, 1 cwt.
Sulphate of potash—nil, 56 lb.
Zinc sulphate—nil, 7 lb.
Copper sulphate—nil, 7 lb.

Blood manure was used in preference to a more soluble nitrogenous fertilizer in order to obviate, as far as possible, the loss of nitrogen by leaching. The dressing used contained an amount of nitrogen equivalent to an application of 94 lb. of nitrate of soda per acre.

These fertilizers were applied in all possible combinations giving 64 treatments. Algerian oats were sown at the rate of 60 lb. per acre.

The fertilizer and oats were broadcast by hand in plots measuring 20 by 25 links on the cultipacked surface of the soil. A disc drill was then run over the plots to cover the seed. Carry-over of seed and fertilizer by the drill from one plot to the next was negligible.

The plots were sown on May 26, 1944, and were inspected at frequent intervals during the growing period. Samples were collected on August 29 when the plants were at the early vegetative stage, on October 10 at the pre-flowering (ears-peeping) stage, and again at maturity on November 28. These were taken by placing two frames measuring 5 by $2\frac{1}{2}$ links on each plot in turn and pulling up by the roots all plants within the frames.

The plants and tillers were counted in each of the samples taken at the vegetative and pre-flowering stages, the roots were then cut off and discarded, and yields were determined after the samples had been dried in an electric oven at 95°C. for 24 hours.

Samples taken at maturity were air-dried, and the number of plants, tillers, and heads were counted. The roots were cut off and discarded and the samples were weighed. The grain was then threshed out, cleaned by hand and weighed, and 1,000-grain weights were determined. Straw yields were estimated by difference.

## 4. Results.

### (i) *Germination and Survival.*

Germination and establishment were satisfactory and were not affected by any of the 64 treatments. On August 29 the mean number of plants per square link was 7·97, or 69·9 per cent. of the viable seeds sown.

Table 1.—Showing the Establishment and Survival of Algerian Oats.

| Number of Viable Seeds Sown per Sq. Link. | Establishment, August 29. | | Survival, November 28. | | |
|---|---|---|---|---|---|
| | Number of Plants per Sq. Link. | Viable Seeds. | —— | Number of Plants per Sq. Link. | Established Plants. |
| | | % | | | % |
| 11·40 | 7·97 | 69·91 | Without superphosphate .. | 5·77 | 72·4 |
| | | | Superphosphate 1 cwt. .. | 7·14 | 89·6 |
| | | | S.E. .. .. .. .. | ±0·364 | |

The survival rate of the plants during the early growing period was the same with all treatments, but between October 10 and the date of harvest there was some mortality among plants grown in the absence of phosphate (Table 1). Without phosphate 72·4 per cent. of the established plants survived until maturity; in the presence of 1 cwt.

of superphosphate 89·6 per cent. survived. The rate of survival was not greater in the plots which were treated with superphosphate at levels higher than 1 cwt. per acre.

(ii) *Development and Yield.*

The plants showed a marked and early response to phosphate, and zinc in the presence of phosphate had a very beneficial effect. Blood manure, in the presence of phosphate, increased the vegetative growth, but did not significantly affect grain production. Neither potash nor copper affected the growth of the plants at any stage of development.

Plants grown without phosphate developed symptoms soon after germination, and these symptoms persisted throughout the growing period. The plants remained stunted. The leaves were hard and leathery, and erect; they were dark-green and frequently tinged with red or purple (Plate 10 and Plate 11, Fig. 1). Guyra oats and Ford wheat in another experiment were affected similarly (Plate 11, Fig. 2, and Plate 12). No tillers were formed and each plant produced one small head containing one under-sized grain (Table 2). Yields were extremely low; less than one bushel of grain was obtained per acre (Table 3).

TABLE 2.—SHOWING THE MAIN EFFECT OF DIFFERENT QUANTITIES OF SUPERPHOSPHATE ON THE CENSUS FEATURES OF ALGERIAN OATS AT MATURITY.

*Means of 16 Plots.*

| — | Superphosphate | | | | S.E. | Percentage Increase between— | |
|---|---|---|---|---|---|---|---|
| | Nil. | 1 cwt. | 2 cwt. | 4 cwt. | | Nil and 1 cwt. | 1 cwt. and 2 cwt. |
| Number of plants per 25 sq. links .. | 144·3 | 178·6 | 199·3 | 161·5 | ±9·09† | 23·8 | 11·6 |
| Number of tillers per 25 sq. links .. | 148·5 | 264·4 | 317·5 | 286·8 | ±9·08† | 78·1 | 20·1 |
| Number of heads per 25 sq. links .. | 124·6 | 229·4 | 269·4 | 259·4 | ±8·99† | 84·1 | 17·4 |
| Percentage head-bearing tillers .. | 83·9 | 86·8 | 84·9 | 90·4 | .. | .. | .. |
| Weight of grain per head (g.)* .. | 0·03 | 0·46 | 0·51 | 0·54 | .. | 1,433·3 | 10·9 |
| Weight of 1,000 grains (g.) .. | 21·30 | 30·14 | 31·20 | 32·51 | ±0·318† | 41·5 | 3·5 |
| Number of grains per head* .. | 1·4 | 15·3 | 16·3 | 16·6 | .. | 992·9 | 6·5 |
| Weight of grain per 25 sq. links (g.) | 4·0 | 106·2 | 136·6 | 139·0 | ±5·85‡ | 2,555·0 | 28·6 |
| Percentage grain in total yield .. | 13·1 | 35·1 | 36·9 | 38·3 | .. | .. | .. |

* Calculated.

† Treatment significant at 1 per cent.

‡ "Superphosphate, Nil" omitted from statistical analysis; treatment significant at 1 per cent.

In the presence of superphosphate the plants grew vigorously and produced good yields. The response to 1 cwt. of superphosphate was pronounced, but maximum yields of grain and straw were obtained with 2 cwt. superphosphate (Table 3) (Plate 11, Fig. 1). There was no further increase in yield with 4 cwt. of superphosphate.

TABLE 3.—SHOWING THE MAIN EFFECT OF SUPERPHOSPHATE ON THE YIELD OF ALGERIAN OATS AT THREE STAGES OF DEVELOPMENT.

*Means of 16 Plots.*

| — | Superphosphate. | | | | S.E.† |
|---|---|---|---|---|---|
| | Nil. | 1 cwt. | 2 cwt. | 4 cwt. | |
| Yield per acre at— | | | | | |
| Vegetative stage, August 29 (cwt.)* | 0·3 | 3·1 | 3·7 | 3·4 | ±0·15 |
| Pre-flowering stage, October 10 (cwt.)* .. .. .. | 0·6 | 11·2 | 13·3 | 13·9 | ±0·73‡ |
| Maturity, November 28— | | | | | |
| Grain (bushels) .. .. | 0·9 | 23·4 | 30·1 | 30·6 | ±1·29‡ |
| Straw (cwt.) .. .. | 2·1 | 15·5 | 18·4 | 17·6 | ±0·71§ |
| Total yield (cwt.) .. .. | 2·4 | 23·8 | 29·2 | 28·6 | ±0·92‡ |

* Oven-dried.

† "Superphosphate, Nil" omitted from analysis of variance.

‡ Treatment significant at 1 per cent.

§ Treatment significant at 5 per cent.

The first increment (1 cwt.) of superphosphate brought about a large increase in the number of grains per head, and this was mainly responsible for the increase of over 1,000 per cent. in the weight of grain per head (Table 2). An increase of 84 per cent. in the number of heads produced per 25 square links was also an important factor in enhancing the grain production.

The second increment of superphosphate was less marked in its effect. The additional grain produced was due mainly to the effect of this increment on the number of heads produced per 25 square links (Table 2).

Zinc sulphate and blood manure both increased the growth in the early stages of development of the plants (Table 4), but only in the presence of superphosphate. Without phosphate growth was uniformly poor. On August 1, ten weeks after sowing, the effect of blood manure was evident in the field. Plants which received blood manure were a darker green colour and more leafy than those which had no blood manure. Although this advantage was maintained throughout the rest of the growing period, the grain yield was not increased significantly

by this treatment (Table 4). At no stage were any marked symptoms of nitrogen deficiency observed in plants grown in the absence of nitrogenous fertilizer. More acute nitrogen deficiency might be expected, however, in succeeding crops grown on this soil.

TABLE 4.—SHOWING THE MAIN EFFECT OF ZINC SULPHATE AND THE MAIN EFFECT OF BLOOD MANURE IN THE PRESENCE OF SUPERPHOSPHATE ON THE YIELD OF ALGERIAN OATS AT THREE STAGES OF DEVELOPMENT.

*Means of 24 Plots.*

| | Zinc Sulphate. | | Blood Manure. | |
|---|---|---|---|---|
| | Nil. | 7 lb. | Nil. | 1 cwt. |
| Yield per acre at— | | | | |
| Vegetative stage, August 29 (cwt.)* | 3·4 | 3·4 | 2·9 | 3·9‡ |
| Pre-flowering stage, October 10 (cwt.)* | 11·6 | 13·9† | 11·3 | 14·3‡ |
| Maturity, November 28— | | | | |
| Grain (bushels) .. .. | 24·1 | 32·0‡ | 26·7 | 29·4 |
| Straw (cwt.) .. .. .. | 15·7 | 18·6‡ | 16·0 | 18·3‡ |
| Total yield (cwt.) .. .. | 24·3 | 30·1‡ | 25·5 | 28·8‡ |

* Oven-dried.

† Increase significant at 5 per cent.

‡ Increase significant at 1 per cent.

Interaction occurred between blood manure and phosphate only at the pre-flowering stage, and at that period the effect of blood manure was greater in the presence of 1 cwt. of superphosphate than in the presence of 2 cwt. or of 4 cwt. of superphosphate. At maturity, grain yields showed no evidence of first or second order interactions between phosphate, blood manure, and zinc.

A response to zinc was first obtained at the pre-flowering stage (Table 4), but no difference between plants which received zinc and those without it was observed in the field at that time. At maturity, however, the effect of zinc was very obvious, both in the appearance of the plants and in the yields obtained (Table 4) (Plate 11, Fig. 1, and Plate 13).

As the plants which were grown with phosphate but without zinc approached maturity, a light-purple discolouration developed on the lower leaves and on the lower part of the stems. This intensified and spread with increasing age, and at maturity the cluster of dead leaves at the base of the plants had assumed a very dark brown colour. At this stage the upper leaves and sheaths, the stems and the panicle-branches were distinctly red or purple, and the veins of the outer glumes were a bluish-green colour. Plants grown in the presence of zinc were generally taller and showed little or no red, purple, or bluish-green colour; and the lowest leaves were a normal green colour or occasionally light-brown. None of the symptoms characteristic of zinc deficiency in oats and described elsewhere (Piper, 1940; Millikan, 1942) were observed at any stage of growth.

This discolouration of plants grown with phosphate in the absence of zinc was neither reduced nor intensified by adding potash, blood manure, or copper, or any combination of them to the soil. Correction of the condition by zinc was almost complete in the presence of 1 cwt.

TABLE 5.—SHOWING THE MAIN EFFECT OF ZINC SULPHATE IN THE PRESENCE OF SUPERPHOSPHATE ON THE CENSUS FEATURES OF ALGERIAN OATS AT MATURITY.

*Means of 24 Plots.*

| — | Zinc Sulphate. | | S.E. | Percentage Increase due to Zinc. |
|---|---|---|---|---|
| | Nil. | 7 lb. | | |
| Number of plants per 25 sq. links .. | 179·4 | 180·2 | ±6·43 | .. |
| Number of tillers per 25 sq. links .. | 275·0 | 304·2 | ±7·41‡ | 10·6 |
| Number of heads per 25 sq. links .. | 242·2 | 263·3 | ±7·34† | 8·7 |
| Percentage head-bearing tillers .. | 88·1 | 86·6 | .. | .. |
| Weight of grain per head (g.)* .. | 0·45 | 0·55 | .. | 22·2 |
| Weight of 1000 grains (g.) .. .. | 30·90 | 31·67 | ±0·26† | 2·5 |
| Number of grains per head .. | 14·6 | 17·4 | .. | 19·2 |
| Weight of grain per 25 sq. links (g.) | 109·3 | 145·3 | ±4·77‡ | 32·9 |
| Percentage grain in total yield .. | 35·4 | 37·6 | .. | .. |

* Calculated.
† Increase significant at 5 per cent.
‡ Increase significant at 1 per cent.

of superphosphate. In the presence of larger quantities of superphosphate (2 cwt. or 4 cwt.) a greater discolouration of this sort was apparent, although the colour was much less intense than it was with similar dressings of superphosphate applied in the absence of zinc.

The striking increase in grain yield which followed the application of zinc sulphate was the result mainly of an increase in the number of grains produced per head (Table 5). Small increases also occurred in the number of heads produced per 25 square links and in the size of the individual grains.

TABLE 6.—SHOWING THE INDIVIDUAL AND COMBINED EFFECTS OF ZINC SULPHATE AND DIFFERENT QUANTITIES OF SUPERPHOSPHATE ON THE GRAIN YIELD OF ALGERIAN OATS.

*Means of 4 Plots. Bushels per acre.*

| — | Superphosphate. | | | |
|---|---|---|---|---|
| | Nil. | 1 cwt. | 2 cwt. | 4 cwt. |
| Zinc sulphate—nil .. .. .. | 0·7 | 16·4 | 23·7 | 26·0 |
| Zinc sulphate—7 lb. .. .. | 0·8 | 26·3 | 33·5 | 34·6 |
| | | (S.E. ± 2·58)* | | |

* "Superphosphate, Nil" omitted from analysis of variance.

The data set out in Tables 3 and 4 render apparent the main effects of the various fertilizers. The individual and combined effects of phosphate and of zinc on the yield of grain are shown in Table 6. Phosphate was obviously the primary limiting factor, and the effect of the addition of phosphate was independent of the effect of zinc (Table 6). The increase in grain yield obtained when the level of superphosphate was increased from 1 cwt. to 2 cwt. was the same in the absence of zinc (7·3 bushels) as it was in the presence of zinc (7·2 bushels); and the increase due to zinc was the same in the presence of 1 cwt. of superphosphate (9·9 bushels) as it was in the presence of 2 cwt. of superphosphate (9·8 bushels). The effect of zinc at each level of phosphate was as great or greater than an increment of an additional 1 cwt. of superphosphate. Total yields were affected similarly.

## 5. Experiments with Pasture Species.

The results obtained during the first year from several factorial experiments with pasture species provided further evidence of the occurrence of trace element deficiencies.

Subterranean clover showed a significant response to the application of zinc sulphate. Copper sulphate further increased the yield of this species (3 strains) in the presence but not in the absence of zinc. In October, the flowering of subterranean clover was observed to be more profuse in the presence of zinc and copper, and burrs and seeds were produced in considerably greater number.

Table 7.—Showing the Effect of Zinc and Copper on the Seed Production of Subterranean Clover.

*Number of Seeds per Square Link.*

| | Zinc Sulphate per Acre. | | Mean (16 plots), (S.E. ± 0·758). |
|---|---|---|---|
| | Nil. | 7 lb. | |
| Copper sulphate, per acre—nil .. .. | 2·87 | 3·88 | 3·38 |
| Copper sulphate, per acre—7 lb. .. | 1·84 | 10·09 | 5·97 |
| Copper sulphate, per acre—14 lb. .. | 1·41 | 8·90 | 5·16 |
| | (S.E. ± 1·072)* | | |
| Mean (24 plots), (S.E. ± 0·619)† .. | 2·04 | 7·62 | .. |

* Interaction significant at 1 per cent.
† Treatment significant at 1 per cent.

The effect of zinc and copper on the seed production of subterranean clover in a factorial experiment is shown in Table 7. The number of burrs produced per square link was affected in a similar manner. The number of plants which became established and which survived to maturity was not affected by the addition of these elements, and the average number of seeds per burr remained constant with all treatments. The effect of zinc and copper on the seed production was solely due to their effect on the number of burrs produced per plant.

Lucerne, in the past, has shown more promise than other legumes grown on the heath sands, and this may have been due, in part at least, to the greater ability of this species to withstand conditions of zinc deficiency. No response was observed to follow the application of zinc, but the addition of copper alone caused a significant increase in the yield. There was no interaction between zinc and copper applied to lucerne.

Yields of subterranean clover and lucerne, typical of those obtained in the first year in a number of experiments, are shown in Tables 8 and 9.

Table 8.—Showing the Effect of Zinc and Copper on the Yield of Subterranean Clover (Bacchus Marsh) in a Mixed Pasture in the First Year.

*Oven-dry Weight in lb. per acre.*

| — | Zinc Sulphate per Acre. | | Mean (20 plots), (S.E. ± 22·6). |
|---|---|---|---|
| | Nil. | 7 lb. | |
| Copper sulphate, per acre—nil .. .. | 132·3 | 249·8 | 191·1 |
| Copper sulphate, per acre—7 lb. .. .. | 133·4 | 342·4 | 237·9 |
| Copper sulphate, per acre—14 lb. .. | 108·3 | 348·6 | 228·5 |
| | (S.E. ± 31·97) | | |
| Mean (30 plots), (S.E. ± 18·4)* .. | 124·7 | 313·6 | |

* Treatment significant at 1 per cent.

Table 9.—Showing the Effect of Zinc and Copper on the Yield of Lucerne in a Mixed Pasture in the First Year.

*Oven-dry Weight in lb. per acre.*

| — | Zinc Sulphate per Acre. | | Mean (20 plots) (S.E. ± 5·89).* |
|---|---|---|---|
| | Nil. | 7 lb. | |
| Copper sulphate, per acre—nil .. .. | 67·4 | 55·2 | 61·3 |
| Copper sulphate, per acre—7 lb. .. .. | 80·8 | 78·6 | 79·7 |
| Copper sulphate, per acre—14 lb. .. | 93·6 | 70·9 | 82·3 |
| | (S.E. ± 8·33) | | |
| Mean (30 plots), (S.E. ± 4·81) .. | 80·6 | 68·2 | .. |

* Treatment significant at 5 per cent.

Phosphate was again the primary limiting factor, and in its absence subterranean clover, lucerne, and several other legumes and grasses remained extremely dwarfed. Potash did not affect the growth either of the grasses or of the legumes. Manganese and gypsum, however, both appeared to have a beneficial effect on the growth of subterranean clover, but the responses were obtained in the absence of zinc and copper and further investigation into these effects is necessary.

## 6. Discussion.

In the soil survey of the Hundreds of Laffer and Willalooka in the Ninety-mile Desert in South Australia (Taylor, 1933), attention was drawn to the extreme poverty of the heath sands, the phosphoric acid content of which was shown to be in the neighbourhood of 0·005 per cent., a figure low even for an Australian soil. In the experiments described here the extremely low phosphoric acid content was reflected in the extraordinarily poor development of cultivated plants sown without phosphate. The response to the addition of superphosphate was marked, and a dressing of 2 cwt. of superphosphate per acre gave maximum yields.

A deficiency of zinc and copper in this soil was revealed. The responses to the application of these elements depended upon the species of plant concerned. Oats benefited markedly from an application of zinc; lucerne responded to copper; and the yield and particularly the seed production of subterranean clover were improved by the addition of zinc and copper together. Responses to these elements were obtained only in the presence of superphosphate.

The grain production of oats was increased very considerably by the application of zinc sulphate, and the effects of zinc and the different levels of superphosphate on the grain yield were independent of one another, and additive. Zinc sulphate at the rate of 7 lb. per acre increased the grain yield by almost 10 bushels per acre whether superphosphate had been applied at 1 cwt., 2 cwt., or 4 cwt. per acre.

None of the lesions described elsewhere (Piper, 1940; Millikan, 1942) as characteristic of zinc deficiency were observed with any treatment at any stage of growth. Similarly, leaf symptoms characteristic of zinc deficiency were not observed on cereals grown on Wimmera black soil (Millikan, 1938; Millikan, 1942) or on Robe calcareous sand (Riceman and Anderson, 1943) even when yields were appreciably increased by the application of zinc.

Some relationship appeared to exist between the effects of zinc and phosphorus on the plants. The brown, purple, and bluish-green discolouration, indicative of a disordered metabolism which can be brought about by phosphorus deficiency, developed with equal intensity at all levels of phosphate in the absence of zinc. The addition of zinc to the manurial dressing reduced this discolouration very obviously; almost completely at the lowest phosphate level, and less completely at the higher phosphate levels. The quantity of zinc applied may have been insufficient to satisfy completely the requirements of the plants grown with even the lowest level of phosphate, or alternatively, the assimilation of zinc may have been complicated by the higher phosphate concentrations. Yield responses to zinc and to phosphate were, however, quite independent of one another. The nitrogenous fertilizer brought about a slight deepening of the green colour of the plants and increased the weight of the straw produced, but did not affect the grain yield significantly. As the soils were relatively high in potassium (Taylor, 1933) no response supervened on the addition of sulphate of potash. No combination of nitrogen, potassium, or copper reduced the discolouration of the plants grown with phosphate.

Other evidence has been obtained suggesting the occurrence of a relationship between phosphorus and zinc in which, however, the degree of zinc deficiency was concerned rather than the degree of phosphorus deficiency. West (1938) suggested that mottle leaf lesions which occurred in citrus grown on plots heavily manured with superphosphate were induced by the phosphate ion. These, he showed, could be cured by the application of zinc. Chapman *et al.* (1937) found that a mottling of citrus leaves due to zinc deficiency was accentuated when the supply of phosphate in the nutrient medium was increased, and considered the effect to be due either to decreased availability of zinc within the plant or to decreased solubility of zinc in the culture solution. Millikan (1940) considered that the application of superphosphate to wheat grown on Wimmera black soil increased the zinc requirement of the plant, and found that a direct relationship existed in the plant between the concentrations of phosphorus and zinc. Piper and Walkley (1943) also found a significant correlation between the amounts of phosphorus and zinc present in fifteen samples of Algerian oats harvested at the flowering stage. These authors suggested, however, that this might be expected as a result of manuring with superphosphate, since it had been shown by Walkley (1940) that the ratio of phosphorus to zinc in Australian superphosphate was of the same order as that in cereals.

The occurrence of a marked discolouration, similar to that described in this paper, in the first crops grown on recently cleared land has been reported from a number of widely separated localities in the Ninety-mile Desert. It may thus be implied that responses to zinc will be general on these soils.

Throughout the growing season the persistence of plants starved in respect of phosphate was remarkable. In spite of their unhealthy condition and very small size, the majority of plants of all types survived to maturity without additional phosphate. Census studies on oats revealed that the predominating effect of the first increment of superphosphate (1 cwt.) was to increase the number of grains per head while a second increment of a similar amount increased the number of heads produced. With the first increment of superphosphate the size of grain was increased and grain yield was augmented to a greater extent than straw yield. With additional increments of superphosphate grain size was increased further and the observed increases in the yield of grain and of straw were more nearly proportional to one another. Similar results have been observed with other cereals (Russell, 1932). Zinc applied at each level of phosphate increased the number of grains per head.

Copper did not affect the yield of oats in this experiment, but evidence obtained from the pasture experiments and from field observations elsewhere suggests that the amount of copper available in these soils might be sub-optimal for the maximum development of leguminous pasture species. On the heath sands the extent to which oats and other cereals might benefit from dressings of copper sulphate when grown under different circumstances is yet to be determined.

The yield of subterranean clover in the first year was increased by the application of zinc sulphate, and further benefit was derived from the application of copper in addition to zinc. Copper applied without

zinc had no effect. The effect of zinc and copper on the seed production of this species was of greater consequence. The seed produced by plants grown with phosphate alone was barely sufficient to reproduce the sparse population which survived during the year of sowing. With the addition of zinc and copper 3·5 times the number of seeds was produced.

The yield of lucerne was increased in the first year by a dressing of copper sulphate, but zinc sulphate had no effect on this species either in the presence or absence of copper. Lucerne appears to be relatively resistant to zinc deficiency (Hoagland *et al.*, 1936, 1937) and this characteristic would partly explain the observed superiority of lucerne compared with other legumes on the heath sands.

It is evident that the poverty of the Laffer sand is largely due to the inadequate available supplies in the soil of phosphate, zinc, and copper. More economic pastoral and agricultural development may almost certainly be achieved in the vast area of closely related soils comprising the Ninety-mile Desert by the employment of adequate dressings of phosphate to which are added the deficient trace elements. The experimental investigation of these limiting factors is proceeding.

## 7. Acknowledgments.

The land and facilities necessary for these experiments were made available by Mr. J. E. Becker, to whom the author wishes to express his sincere thanks. He is also grateful to Mr. J. O. Wilson for the photography and for other assistance, and to Miss Miriam Davies and Miss Marguerite Halls for assistance in the laboratory.

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# Stock and Scion Investigations.

## V. A Nursery Trial with Apple Rootstocks.

*By L. A. Thomas, M.Sc.**

*Summary.*

The apple variety Jonathan was used as a scion on several rootstocks in a nursery trial, where the trees were planted four feet by five feet apart. Of the six rootstocks tested, Merton No. 793 induced the greatest vegetative vigour and the best cropping in the scion over the eight-year period of the trial. The vegetative vigour induced by the other five stocks was in the order from the highest to the lowest, Malling No. XVI., Merton No. 789, local selections D and E, and Northern Spy. Data on the growth, blossoming, and rooting habits of these stocks are presented and their behaviour compared and evaluated.

### 1. Introduction.

This report is an account of the first nursery trial, Trial A, referred to by Dickson and Thomas (1938), which was begun in 1936 and completed in 1944.

These closely planted stock trials were designed to give a quick preliminary indication of the vigour and cropping ability of any particular rootstock, and to provide an inexpensive means of eliminating unsuitable varieties from a bulk of rootstocks.

These trials follow those designed by Tydeman (1940) at East Malling, with alterations in cultural practices to suit Australian conditions.

### 2. Material and Methods.

The rootstocks used in this trial were obtained from several sources; Malling XVI. and the Merton stocks 789 and 793 from East Malling Research Station; stocks E and D are local selections, the former being obtained from Mr. H. St. J. Pratt of the Queensland Department of Agriculture and Stock; and the Northern Spy was that used by a prominent nurseryman. The Merton stocks are immune to woolly aphis and were bred at the John Innes Horticultural Institute, England, by crossing Malling II. x Northern Spy.

All stocks for the trial were produced from stoolbeds, and their propagating ability noted for two or more years. All stocks averaged over 95 per cent. rooted shoots, although stocks E and 789 were not as well rooted as the others.

One-year-old whips were selected from the nursery and transplanted in the winter of 1937 at a spacing of 4 feet by 5 feet, to form a randomized trial. There were six plots each of five units for stocks XVI. and E, and six plots each of four units for stocks D, Spy, 789, and 793. The guard rows were of Granny Smith on stock E.

The trees were grown with a central leader; half the previous season's leader growth was pruned off each winter; lateral growths if longer than about nine inches were pruned to five buds; and all thinning of wood was done in a systematic manner.

An artificial fertilizer mixture made from two parts superphosphate, two parts sulphate of ammonia and one part muriate of potash was applied at the rate of ¼ lb. per tree each year. As a safeguard against Little Leaf and Summer Dieback, zinc sulphate was sprayed on the

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trees in the winter at the rate of 25 lb. per 100 gallons of water and copper sulphate applied as a soil dressing during alternate years.

For the last three years of the trial borax was applied in the calyx and first-cover sprays at 5 lb. per 100 gallons, to overcome drought spot of fruit and "measles" of the wood (1944). Volunteer weed growth was encouraged and chopped in with a rotary hoe.

The annual wood growth was measured, blossom clusters counted before pruning for the first three years after transplanting, all fruits borne were weighed and counted, the weights of the scions determined in 1944, when the trees were cut off at the union with the stock, and the root systems were examined by excavation.

### 3. Results.

The accumulated total shoot growth in metres is shown graphically in Fig. 1, and the pertinent statistical data are presented in Table

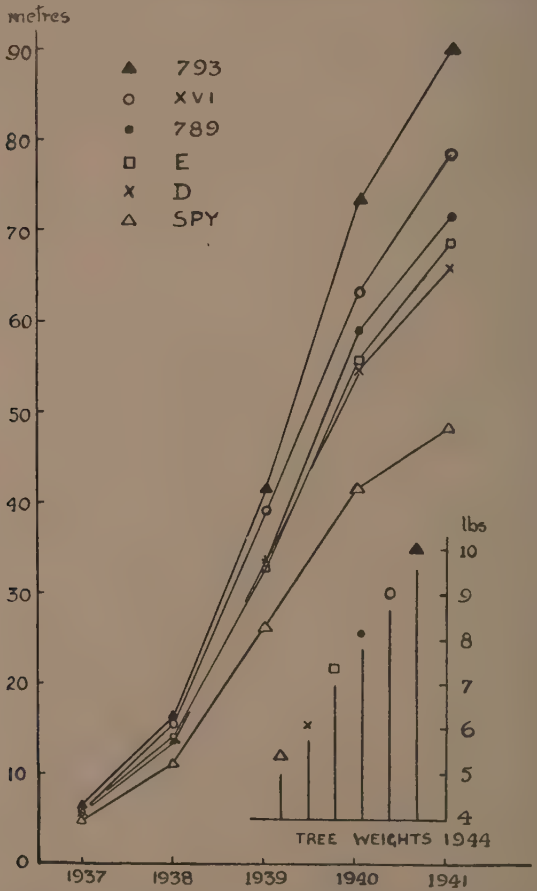


FIG. 1.—Shows the total accumulated growth (in metres) of Jonathan trees for the period 1937 to 1941 on various rootstocks and final tree weights in lb. in 1944.

1. The total accumulated growth in centimetres to the end of each season was analysed, using the standard procedure for randomized block design. By the end of 1941, the majority of the differences between pairs of stocks were significant at the 5 per cent. level at least, and it is likely that the ranking of all stocks at this date is correct.

The critical differences given in the table of means are the least differences between pairs of treatments to be significant at the designated level.

TABLE 1.—TREATMENT MEANS.

| Stock. | 1937. | 1938. | 1939. | 1940. | 1941. |
|---|---|---|---|---|---|
| 793 .. .. | 638 | 1,619 | 4,182 | 7,390 | 9,064 |
| XVI. .. .. | 560 | 1,547 | 3,925 | 6,342 | 7,890 |
| 789 .. .. | 534 | 1,364 | 3,377 | 5,928 | 7,202 |
| E .. .. .. | 568 | 1,390 | 3,285 | 5,606 | 6,910 |
| D .. .. .. | 538 | 1,381 | 3,368 | 5,499 | 6,624 |
| Spy .. .. | 484 | 1,103 | 2,647 | 4,188 | 4,860 |
| Critical Differences— | | | | | |
| 5 per cent. .. .. | 160·4 | 272·4 | 440·4 | 566·8 | 696·9 |
| 1 per cent. .. .. | 218·8 | 371·5 | 600·7 | 773·1 | 950·4 |
| 0·1 per cent. .. | 296·1 | 502·7 | 812·9 | 1,046·2 | 1,286·2 |

It will be seen that Merton 793 as a rootstock had produced about twice as much scion growth as compared with the trees on Northern Spy stocks, and that by 1941, the rate of growth of trees on Spy stocks had decreased when compared with those on 793. Since this date trees on Stocks D and E have also shown a decrease in their rate of growth, as compared with those on stocks 793, 789, and XVI.

The data for scion weights obtained in 1944 are shown in Fig. 1 and in Table 2. The order for scion weight is the same as that determined from total accumulated growth, and serves as a means of further stressing the differences between Spy and the Merton stocks of Spy parentage.

TABLE 2.—TREE WEIGHTS IN LB. IN 1944. MEANS FOR EACH PLOT.

| Stock. | Rows 1. | 2. | 3. | 4. | 5. | 6. | Mean/Tree. |
|---|---|---|---|---|---|---|---|
| 793 .. .. | 9·05 | 10·86 | 10·87 | 8·20 | 9·24 | 9·12 | 9·56 |
| XVI. .. | 10·68 | 8·52 | 9·30 | 7·34 | 7·87 | 8·30 | 8·67 |
| 789 .. .. | 8·69 | 7·99 | 7·61 | 6·67 | 6·44 | 9·16 | 7·76 |
| E .. .. | 7·48 | 7·48 | 7·29 | 6·72 | 5·46 | 7·45 | 6·98 |
| D .. .. | 5·98 | 6·82 | 5·55 | 5·74 | 3·57 | 6·83 | 5·75 |
| Spy .. .. | 6·05 | 5·66 | 6·13 | 4·75 | 3·01 | 4·56 | 5·03 |

Minimum difference between means for significance at 5 per cent. level is 0·92 lb., and at the 1 per cent. level is 1·24 lb.

The numbers of blossom clusters were counted for the three years 1937 to 1939, to gain information as to the early or late fruitfulness induced in the scion by the rootstock. This information is given in

Table 3. It will be noted that trees on Malling XVI., which are slow to come into cropping, have the least number of blossom clusters, whereas trees on Spy have the greatest number, indicating its habit of inducing early fruitfulness in a scion.

TABLE 3.—BLOSSOMING AND CROPPING DATA, 1937-1944.

| Stock. | D. | E. | XVI. | Spy. | 789. | 793. |
|---|---|---|---|---|---|---|
| Average number blossom clusters per tree, 1937–39 | 36·9 | 13·5 | 3·7 | 76·8 | 67·8 | 27·8 |
| Average weight fruit borne (lb. per tree), 1937–44 .. | 32·5 | 35·4 | 39·9 | 40·0 | 75·4 | 78·5 |
| Average weight of ten fruits (lb.), 1937–44 .. .. | 1·98 | 2·23 | 2·29 | 2·0 | 2·14 | 2·28 |
| Estimated crop per acre (bushels), 1937–44—2,178 trees per acre .. .. | 1,792 | 1,929 | 2,169 | 2,180 | 4,104 | 4,272 |

Tydeman (1940) using the scion variety Lane's Prince Albert with these Merton stocks, Spy, and others, showed that the Merton stocks are superior to Spy in promoting scion growth, but do not differ markedly from one another. However, in this experiment, with Jonathan as scion, Merton 793 is shown to be a superior stock when compared with Merton 789, both in relation to growth produced and the amount of crop borne.

The data giving aspects of fruit production in Table 3 present interesting points. The order for fruiting is not the same as that for growth: for instance stock 793, which produced the greatest weight of fruit, has also grown the largest trees. Yet Northern Spy, with the lowest growth, has outyielded trees on three other stocks, D, E, and XVI., which have greater total wood growth. It is recognized, however, that stock XVI., which is classed as very vigorous at East Malling, and is slow in coming to the full bearing stage, may outyield trees on these other rootstocks. With such closely planted trees grown without a supplementary water supply, the leaves often wilted during dry periods, but there was no observable indication of differences in time or severity of wilting due to the several rootstocks.

Because of these dry spells, the average size of the fruit may have been affected, but the figures given in Table 3 indicate that throughout their whole life the trees produced reasonably well sized fruit. The smallest fruits, which were produced by trees on stocks D and Spy, may be attributed to the fact that these stocks had the shallowest and sparsest rooting systems of the series.

The estimated cropping per acre for trees on each rootstock planted at five feet by four feet, serves to emphasize the potentialities of the different rootstocks in relation to crop production. Except in the case of Northern Spy, where 6 per cent. of the total crop was borne in the first four years, the other trees produced these estimated crops during the last four years of the trial.

*Rooting Systems.*

The rooting systems of the several rootstocks have been observed by excavation of the whole root system. In Fig. 2 the rooting systems of adjacent trees on stocks Northern Spy and Merton 793 are shown in plan. Northern Spy shows a typical unbalanced rooting system: the main roots are shallower than those of other stocks, except stock D, most of the roots occurring at from 3 to 6 inches below soil level, with some extremities reaching greater depths. Short fibrous roots developing along the main roots is a character of Spy rootstocks.

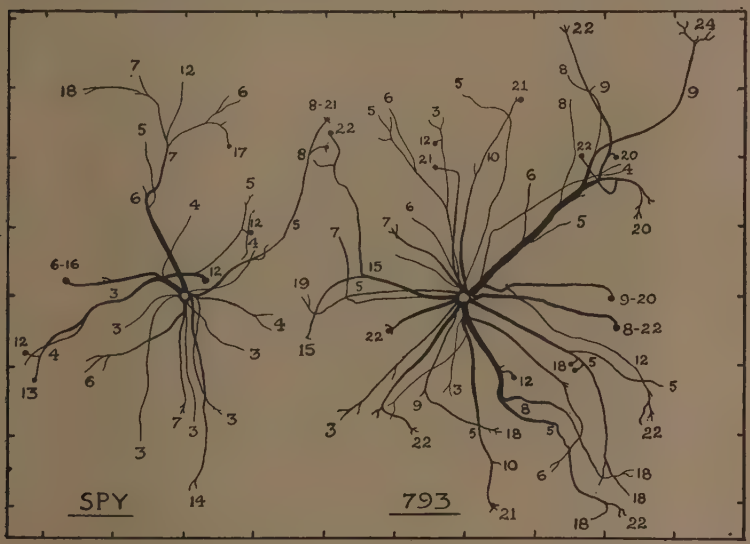


Fig. 2.—Plan of the root system of adjacent trees on Northern Spy and Merton 793 rootstocks. Scale 1 inch = 1 foot; depth of rooting shown in inches. Black dots indicate where roots descend rapidly to the depths shown.

Stock 793 develops a strong, even rooting system, with the main roots occurring at depths of 6 to 9 inches and descending gradually in most cases to 18 to 24 inches.

Stock D resembles Northern Spy in its habit of rooting and the amount of fibrous root developed.

Malling XVI. is a strongly rooted stock with coarse main roots and comparable to 793.

Stock E has a number of medium sized main roots, penetrating gradually to depths of from 10 to 18 inches. The rooting system is evenly developed with small amount of fibre.

Stock 789 develops a coarse rooting system like 793, but fibrous roots are more plentiful. The main roots do not penetrate as deeply as those of 793, mostly descending at depths of 15 to 20 inches. No roots were observed shallower than six inches.

## 4. Discussion.

It is considered that because the trees in this trial have produced four crops of fruit and thus indicated their cropping habits, and because the trees on stocks D, E, and Spy have shown a decline in their rate of growth, there is sufficient preliminary information to warrant the use of Merton stocks 793 and 789 in further extensive trials with other scion varieties.

The combination of vigour and fruitfulness exhibited by Merton 793 is an outstanding characteristic; much the same can be claimed for Merton 789, and this stock may prove to produce a profitable tree with more vigorous scion varieties, such as Delicious.

A further desirable character of these stocks is their immunity to woolly aphis, and for this reason their propagation should prove to be economical in the southern hemisphere where Northern Spy is commonly used as a stock for this same reason.

## 5. Acknowledgment.

The writer gratefully acknowledges the assistance of Mr. G. A. McIntyre, B.Sc., Dip. Ed., who made the statistical analyses of the data here reported.

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# The Oxidant Effect of Commercial Salt in Fats and Oils.

*By G. Loftus Hills, B.Agr.Sc.,* and J. Conochie, B.Sc.(Agric.)**

*Summary.*

Commercial dairy salt prepared from seawater was found to accelerate the oxidation of dry butterfat, lard, and beef fat, and to a lesser extent of maize oil and peanut oil. Some English and American salts, probably from rock salt, showed less activity. The oxidant action was due to traces of magnesium chloride in the salt. Evidence is presented to show that the magnesium chloride acted as a heterogeneous catalyst. Chlorides of calcium, aluminium, zinc, and beryllium were similarly active. Grinding salt with 0·5 per cent. of sodium carbonate greatly reduced the oxidant action. Although commercial salt in solution accelerated the oxidation of butterfat, neither in incubated butterfat containing dispersed brine nor in cold-stored butter was this acceleration due to magnesium chloride.

## 1. Introduction.

The accelerative action of common salt on the rate of fat oxidation in cold-stored butter has been shown by the work of Wiley (1939), Scheib, Stark, and Guthrie (Guthrie *et al*, 1936; Scheib *et al.*, 1942), and Overman, Garrett and Ruehe (1938). Zinov'ev (1942) found that salt containing 0·01 per cent. of iron accelerated the oxidation of butter. In comparing butters containing dairy salt (probably Australian) and stoved and unstoved vacuum salt, Valentine (1938) found no difference in initial flavour and very little difference in keeping quality.

Lea (1934) found that sodium chloride accelerated oxidation of bacon fat, but after later work (Lea, 1936), in which even 26 per cent. sodium chloride in buffered solution failed to accelerate oxidation of lard, he concluded that the effect had been due to traces of alkali or copper in the salt used. Banks (1937) stated that although common salt can activate a fat oxidase in herring muscle, it causes no acceleration of the oxidation of herring oil in an emulsion stabilized with calcium palmitate. Sandor (1939) in a three-year storage test of butterfat, lard, and pumpkin-seed oil, found that treatment with salt which was then filtered off before storage had no clear effect on subsequent changes.

## 2. Experimental Work.

In concentrated butter, dry butterfat containing 3-5 per cent of fully hydrogenated peanut oil is mixed with 2 per cent. of finely ground salt and 4 per cent. of skim milk powder. Factors affecting the oxidation of this product have been studied by the following method:— Butterfat of initial Lea peroxide value not exceeding 0·3 was melted and mixed with 4 per cent. of fully hydrogenated peanut oil. Solids or liquids to be incorporated in the fat were weighed into 170 ml. brown glass jars; 50 ml. of fat was then added, the jars immersed in iced water and stirred uniformly for a standard time (usually 3 minutes) until the fat solidified sufficiently to hold in suspension any other phase present. Each treatment was duplicated and the duplicates in tables of results in this report are designated A and B. The jars were then placed in an incubator at 40°C. Higher temperatures were not used

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because 40°C. is about the maximum temperature to which concentrated butter is likely to be subjected in practice, and observations here as well as the work of other investigators have shown that misleading results may be obtained by working at widely different temperatures. Also, at 40°C. the fat mixture is sufficiently firm to keep added solids or liquids in suspension, thereby increasing the area of contact.

In early work the samples, after incubation, were examined for Lea (1938) peroxide values and Schibsted (1932) aldehyde values. Incubation periods of 8 to 14 days were then necessary. Later the more sensitive thiocyanate peroxide test (Loftus Hills and Thiel, 1945) was used in conjunction with the Lea test, and it was found possible to reduce the holding period to 3–5 days. By working with low peroxide values, variation in tests due to limitation of oxygen supply was avoided, since sufficient oxygen was initially dissolved in the fat. Degree of oxidized flavour was noted and was nearly always proportional to the measured fat oxidation values.

After removal from the incubator the samples were warmed to 60°C., well mixed and poured in to centrifuge tubes, centrifuged, placed in a bath at 60°C. and the fat then measured out for the oxidation tests. Great care was taken throughout to ensure the cleanliness of glassware, and to reduce to a minimum exposure of the fat to light.

### 3. The Effect of Salt on Fat Oxidation.

In some of the initial tests it was found that commercial dairy-grade salt accelerated the oxidation of dry butterfat. This was not unexpected, in view of the known effect of salt on the oxidation of butter. However, further tests showed that A.R. grade sodium chloride did not increase the rate of oxidation of butterfat. Some results of an oxidation experiment bearing on this point are given in Table 1.

Table 1.—The Effect of Commercial Dairy Salt and A.R. Grade Sodium Chloride on the Oxidation of Dry Butterfat.

| Sample. | | After Twelve Days at 40°C. | | | |
|---|---|---|---|---|---|
| | | Lea Peroxide Value. | | Aldehyde Value. | |
| Control fat | A. | 1·25 | 1·23 | 2·32 | 2·26 |
| | B. | 1·21 | | 2·20 | |
| Fat + 2 per cent. ground commercial salt | A. | 1·76 | 1·68 | 3·24 | 3·45 |
| | B. | 1·60 | | 3·66 | |
| Fat + 2 per cent. ground A.R. sodium chloride | A. | 1·13 | 1·10 | 1·98 | 1·89 |
| | B. | 1·07 | | 1·79 | |

Study of a wider range of salts showed that while all Australian salts tested were markedly oxidant, some samples of English and American commercial salts were much less active. Figures derived from several tests are given in Table 2. Salt used in this and other tests was ground with mortar and pestle. Grinding slightly increased the oxidant effect.

TABLE 2.—THE OXIDANT ACTIVITY OF SOME AUSTRALIAN, ENGLISH, AND AMERICAN SALTS.

| Sample. | After Nine Days at 40°C. | |
|---|---|---|
| | Lea Peroxide Value. | Aldehyde Value. |
| Control fat .. .. .. .. | A. 1·90, B. 1·84 } 1·87 | A. 4·2, B. 3·8 } 4·0 |
| Fat + 5 per cent. Australian commercial salt No. 1 | A. 15·8, B. 17·2 } 16·5 | A. 95, B. 84 } 90 |
| Fat + 5 per cent. English commercial salt No. 1 | A. 2·24, B. 2·10 } 2·17 | A. 5·5, B. 5·0 } 5·3 |
| Fat + 5 per cent. English commercial salt No. 2 | A. 3·49, B. 2·52 } 3·01 | A. 11·8, B. 7·7 } 9·8 |
| Fat + 5 per cent. English commercial salt No. 3 | A. 1·79, B. 1·79 } 1·79 | A. 4·7, B. 3·7 } 4·2 |
| Fat + 5 per cent. American commercial salt No. 1 | A. 2·22, B. 2·18 } 2·20 | A. 6·7, B. 5·8 } 6·3 |

The Australian salt is made by evaporation of seawater, while many English and American brands of salt are derived from underground salt deposits. When active salt was heated to 300°C. or more for three hours, the oxidant activity was reduced or even eliminated, as shown by the results quoted in Table 3.

TABLE 3.—THE EFFECT OF HEATING COMMERCIAL SALT ON ITS OXIDANT ACTIVITY.

| Sample. | After Eight Days at 40°C. | |
|---|---|---|
| | Lea Peroxide Value. | Aldehyde Value. |
| Control fat .. .. .. .. | A. 1·44, B. 1·45, C. 1·45 } 1·45 | A. 2·90, B. 3·06, C. 2·76 } 2·91 |
| Fat + 5 per cent. commercial salt .. | A. 4·45, B. 4·15 } 4·30 | A. 16·6, B. 16·1 } 16·4 |
| Fat + 5 per cent. commercial salt heated to 500°C. for three hours .. .. .. | A. 1·39, B. 1·37 } 1·38 | A. 3·01, B. 2·71 } 2·86 |

The copper content of the salt used in this test was 0·9 p.p.m., and the iron content 12 p.p.m. Heating the salt did not change these values. The elimination of oxidant activity by heating therefore suggested that traces of heavy metals in the salt were not the source of the activity. Confirmation of this was obtained when it was found that addition of Cu and Fe in these proportions to A.R. sodium chloride did not appreciably alter its oxidant activity in dry butterfat. The oxidant effect was greatly reduced by extracting the commercial salt with alcohol, and to a lesser extent by brine washing, and extraction with acetone. Extraction with carbon tetrachloride or with petroleum ether was not effective.

Harvey (1925) had found an oxidant substance, probably organic in nature, in seawater. Mixed salt was therefore prepared by evaporation of seawater to dryness, and this proved to be strongly oxidant.

In attempting to concentrate the oxidant substance, commercial salt solution was made sufficiently alkaline to precipitate magnesium and calcium salts. This precipitate was filtered off, and the salt prepared by evaporation of the filtrate showed greatly reduced oxidant activity in butterfat. The precipitate was dissolved in hydrochloric acid. When this solution was neutralized and added to a solution of pure sodium chloride in correct proportion, the salt finally obtained by evaporation and grinding was more active than the original commercial salt. If the alkaline precipitate was dissolved in hydrochloric acid, neutralized, evaporated, and extracted with acetone, the acetone extract was strongly oxidant. Up to this point the evidence was consistent with the supposition that the oxidant catalyst was organic in nature. The acetone extract proved, however, to contain much copper, and a quantitative test, the results of which are given in Table 4, showed that the acetone extract accounted for relatively little of the oxidant activity, which was concentrated in the alkaline earth chlorides.

Table 4.—Quantitative Distribution of the Oxidant Effect at Various Stages in the Alkali Precipitation, Acetone Extraction Procedure.

| Sample. | After Twelve Days at 40°C. | |
|---|---|---|
| | Lea Peroxide Value. | Aldehyde Value. |
| Control fat .. .. .. .. | A. 1·04, B. 1·05 } 1·05 | A. 1·43, B. 1·45 } 1·44 |
| Fat + 5 per cent. commercial salt .. | A. 2·59, B. 2·43 } 2·51 | A. 9·95, B. 8·33 } 9·14 |
| Fat + 5 per cent. commercial salt purified by alkali precipitation .. .. .. | A. 1·31, B. 1·23 } 1·27 | A. 2·69, B. 3·04 } 2·87 |
| Fat + 5 per cent. A.R. NaCl with neutralized alkali precipitate .. .. .. | A. 5·23, B. 8·25 } 6·74 | A. 23·0, B. 26·6 } 24·8 |
| Fat + 5 per cent. A.R. NaCl with neutralized alkali precipitate after acetone extraction .. | A. 4·51, B. 4·81 } 4·66 | A. 15·8, B. 18·2 } 17·0 |
| Fat + 5 per cent. A.R. salt with acetone extract | A. 1·63, B. 1·77 } 1·70 | A. 4·08, B. 4·40 } 4·24 |

The previous observations including inactivation by heat and extraction with alcohol were quite consistent with magnesium chloride being the source of oxidant activity, and further work soon established this with certainty. The salt used was found to contain 0·09 per cent. of magnesium chloride and 0·25 per cent. of calcium sulphate. Magnesium chloride was prepared by dissolving pure magnesium in redistilled hydrochloric acid, precipitating with hydrogen chloride, washing the precipitate with redistilled acid, and drying in a vacuum oven at 100°C. A.R. grade magnesium chloride was purified by the

same procedure. These two lots of magnesium chloride were added separately to A.R. sodium chloride in solution, and salts prepared by evaporation and grinding. These were added to butterfat in an oxidation experiment, the results of which are shown in Table 5. Another similar experiment in which sodium chloride containing

TABLE 5.—THE OXIDANT EFFECT OF MAGNESIUM CHLORIDE ADDED TO A.R. SODIUM CHLORIDE.

| Sample. | After Five Days at 40°C. | |
|---|---|---|
| | Thiocyanate Peroxide Value. | Lea Peroxide Value. |
| Control fat .. .. .. .. | A. 2·80, B. 2·62 } 2·71 | A. 1·18, B. 1·03 } 1·11 |
| Fat + 5 per cent. A.R. sodium chloride .. | A. 2·76, B. 2·85 } 2·81 | A. 1·13, B. 1·20 } 1·17 |
| Fat + 5 per cent. A.R. sodium chloride containing 0·5 per cent. $MgCl_2$ (from metal) .. | A. 6·41, B. 6·33 } 6·37 | A. 2·62, B. — } 2·62 |
| Fat + 5 per cent. A.R. sodium chloride containing 0·5 per cent. $MgCl_2$ (from A.R. $MgCl_2$) .. .. .. .. .. | A. 5·63, B. 5·63 } 5·63 | A. 2·59, B. 2·39 } 2·49 |
| Control fat .. .. .. .. | A. 2·34, B. 2·31 } 2·32 | |
| Fat + 5 per cent. A.R. sodium chloride .. | A. 2·38, B. 2·41 } 2·40 | |
| Fat + 5 per cent. A.R. sodium chloride containing 0·04 per cent. $MgCl_2$ .. .. .. | 3·30 | |
| Fat + 5 per cent. A.R. sodium choride containing 0·09 per cent. $MgCl_2$ .. .. .. | 4·08 | |
| Fat + 5 per cent. A.R. sodium chloride containing 0·5 per cent. $MgCl_2$ .. .. .. | 3·85 | |
| Fat + 5 per cent. A.R. sodium chloride containing 0·09 per cent. $MgCl_2$ and 0·25 per cent. $CaSO_4$ | 4·18 | |
| Fat + 5 per cent. A.R. sodium chloride containing 0·09 per cent. $MgCl_2$, 0.25 per cent. $CaSO_4$, and 0·5 p.p.m. Cu .. .. .. .. | 4·51 | |
| Fat + 5 per cent. commercial salt .. .. | 4·13 | |

various quantities of magnesium chloride and an artificial commercial salt containing added magnesium chloride, calcium sulphate, and cupric chloride were compared in activity with commercial salt is also reported in Table 5. A.R. sodium chloride to which small amounts of magnesium chloride were added had similar oxidant activity to commercial salt. The artificial salt proved a little more active than the commercial salt, perhaps because the copper was in a more active form. In commercial salt the copper may be present partly as particles of Monel metal derived from the plant.

Several further tests with varying concentrations up to 80 per cent. of magnesium chloride in the salt showed that little further activity was conferred by increasing the concentration above 0·1 per cent., although there was a tendency towards a maximum with 50–70 per cent. magnesium chloride.

Berkman, Morrell, and Egloff (1940) mention the use of magnesium chloride as a catalyst in hydrogenation and polymerization reactions, in the oxidation of alcohol, and in other reactions, but no previous mention has been found of its catalytic activity in the oxidation of fats and oils.

The chlorides of several related metals were found to be more strongly oxidant. Table 6 shows the activities of preparations made from A.R. sodium chloride by addition of the chlorides of beryllium, aluminium, zinc, calcium, and magnesium respectively. The prepared salts were obtained from solution by evaporation, ground and added to butterfat in 5 per cent. concentration.

TABLE 6.—THE OXIDANT ACTIVITY IN BUTTERFAT OF SEVERAL METALLIC CHLORIDES ADDED TO SODIUM CHLORIDE.

| Sample. | After Five Days at 40°C. | |
|---|---|---|
| | Thiocyanate Peroxide Value. | Lea Peroxide Value. |
| Fat + 5 per cent. A.R. sodium chloride .. A. | 1·21 | 0·57 |
| B. | 1·21 | 0·57 |
| | 1·21 | 0·57 |
| Fat + 5 per cent. A.R. NaCl containing 0·1 per cent. $BeCl_2$ .. .. .. .. A. | 2·53 | 1·07 |
| B. | 2·53 | 1·08 |
| | 2·53 | 1·08 |
| Fat + 5 per cent. A.R. NaCl containing 1·0 per cent. $BeCl_2$ .. .. .. A. | 3·13 | 1·40 |
| B. | 3·13 | 1·41 |
| | 3·13 | 1·41 |
| Fat + 5 per cent. A.R. NaCl containing 0·1 per cent. $AlCl_3$ .. .. .. A. | 3·24 | 1·47 |
| B. | 3·24 | 1·48 |
| | 3·24 | 1·48 |
| Fat + 5 per cent. A.R. NaCl containing 1·0 per cent. $AlCl_3$ .. .. .. A. | 0·27 | 0·24 |
| B. | *0·27 | *0·27 |
| | 0·27 | *0·26 |
| Fat + 5 per cent. A.R. NaCl containing 0·1 per cent. $ZnCl_2$ .. .. .. A. | 3·21 | 1·50 |
| B. | 3·24 | 1·48 |
| | 3·23 | 1·49 |
| Fat + 5 per cent. A.R. NaCl containing 1·0 per cent. $ZnCl_2$ .. .. .. A. | 3·18 | 1·53 |
| B. | 3·27 | 1·47 |
| | 3·23 | 1·50 |
| Fat + 5 per cent. A.R. NaCl containing 0·1 per cent. $CaCl_2$ .. .. .. A. | 2·95 | 1·28 |
| B. | 2·95 | 1·30 |
| | 2·95 | 1·29 |
| Fat + 5 per cent. A.R. NaCl containing 1·0 per cent. $CaCl_2$ .. .. .. A. | 3·01 | 1·29 |
| B. | 2·95 | 1·32 |
| | 2·98 | 1·31 |
| Fat + 5 per cent. A.R. NaCl containing 0·1 per cent. $MgCl_2$ .. .. .. A. | 2·07 | 0·94 |
| B. | 1·94 | 0·90 |
| | 2·01 | 0·92 |
| Fat + 5 per cent. A.R. NaCl containing 1·0 per cent. $MgCl_2$ .. .. .. A. | 2·24 | 0·99 |
| B. | 2·32 | 1·04 |
| | 2·28 | 1·02 |

* These fats were bleached.

The activity of calcium chloride may be of practical importance where refrigerating brine gains access to fat foods, or when traces of calcium chloride occur in commercial salt. The increased activity when the alkali precipitate from commercial salt is dissolved in hydrochloric acid, neutralized, and added to A.R. sodium chloride (Table 4), is probably due to formation of calcium chloride from calcium sulphate in the original salt. Aluminium chloride could conceivably gain access to salted fat foods when salt comes in contact with aluminium vessels used in their preparation. Zinc chloride may gain contact with fat through incomplete removal of soldering flux containing zinc chloride.

Of the other halides of magnesium, the bromide was about as active as the chloride, both in the dry state on sodium chloride, and in 20 per cent. solution. The iodide was less active in solution and anti-oxidant in the dry state; the salt was pink in colour due to liberation of iodine from the magnesium iodide; this iodine may account for the anti-oxidant effect.

### 4. Oxidation of Fats other than Butterfat by Commercial Salt.

Oxidation tests with other fats reported in Table 7 showed that lard and beef fat are strongly affected by traces of magnesium chloride in salt, but that maize and peanut oils are only slightly affected. Lack of dispersion of the salt through these oils may account in part for this, since when maize oil was solidified with hydrogenated peanut oil the salt had more effect. On the other hand, butterfat without hydrogenated peanut oil, from which the salt would settle at 40°C., was oxidized almost as much by commercial salt as the normal butterfat containing hydrogenated peanut oil.

TABLE 7.—THE OXIDANT EFFECT OF COMMERCIAL SALT ON OTHER FATS. INITIAL LEA PEROXIDE VALUES WERE BEEF FAT 0·52, MAIZE OIL 4·1, AND PEANUT OIL 10·4.

| Sample. | After Four Days at 40°C. | |
|---|---|---|
| | Thiocyanate Peroxide Value. | Lea Peroxide Value. |
| Beef fat .. .. .. .. .. A. | 1·32 | 0·52 |
| B. | 1·29 | 1·31 | 0·51 | 0·52 |
| Beef fat + 5 per cent. A.R. sodium chloride .. A. | 1·36 | 0·55 |
| B. | 1·35 | 1·36 | 0·52 | 0·53 |
| Beef fat + 5 per cent. commercial salt .. A. | 2·53 | 1·04 |
| B. | 2·49 | 2·51 | 1·00 | 1·02 |
| Maize oil .. .. .. .. A. | 65 | 22·6 |
| B. | 71 | 68 | 22·6 | 22·6 |
| Maize oil + 5 per cent. A.R. sodium chloride A. | 65 | 23·0 |
| B. | 68 | 67 | 22·4 | 22·7 |
| Maize oil + 5 per cent. commercial salt .. A. | 79 | 26·0 |
| B. | 77 | 78 | 26·6 | 26·3 |
| Peanut oil .. .. .. .. A. | 61 | 21·6 |
| B. | 58 | 60 | 21·4 | 21·5 |
| Peanut oil + 5 per cent. A.R. sodium chloride A. | 50 | 19·9 |
| B. | 57 | 54 | 21·1 | 20·5 |
| Peanut oil + 5 per cent. commercial salt .. A. | 66 | 23·1 |
| B. | 67 | 67 | 23·4 | 23·3 |

| Sample. | After Thirteen Days at 40°C. | |
|---|---|---|
| | Thiocyanate Peroxide Value. | Lea Peroxide Value. |
| Lard .. .. .. .. .. A. | 2·34 | 1·14 |
| B. | 2·22 | 2·28 | 1·15 | 1·15 |
| Lard + 5 per cent. A.R. sodium chloride .. | 3·12 | 1·36 |
| Lard + 5 per cent. commercial salt .. .. | 40 | 19·5 |

### 5. Treatment of Salt.

It has been mentioned that the oxidant effect of salt can be reduced by heating and by brine or alcohol extraction. The oxidant effect can also be greatly reduced by grinding the salt with sodium carbonate. At first a little moisture was also added and the salt stirred and heated to 200°C. Later it was found that fine grindng alone is sufficient to bring about the reaction between the sodium carbonate and magnesium chloride. The effect of this treatment and the magnitude of the residual oxidant effect is shown in Table 8.

Table 8.—The Effect on Oxidant Activity of Commercial Salt of Grinding with Sodium Carbonate.

| Sample. | After Four Days at 40°C. | |
|---|---|---|
| | Thiocyanate Peroxide Value. | Lea Peroxide Value. |
| Control fat .. .. .. .. | A. 0·68, B. 0·67 } 0·68 | A. 0·36, B. 0·38 } 0·37 |
| Fat + 5 per cent. commercial salt .. | A. 2·00, B. 2·01 } 2·01 | A. 1·13, B. 1·08 } 1·11 |
| Fat + 5 per cent. commercial salt ground with 0·5 per cent. sodium carbonate .. .. | A. 0·80, B. 0·80 } 0·80 | A. 0·41, B. 0·52 } 0·47 |

Disodium phosphate gave similar results and sodium bicarbonate was somewhat less effective. Salt used for the manufacture of concentrated butter in Australia is now ground with sodium carbonate.

### 6. Mode of Action of Magnesium Chloride.

Magnesium chloride, being much more soluble than sodium chloride, probably exists in commercial salt chiefly as a film on the sodium chloride crystals. Such a physical condition would favour either solution of the magnesium chloride in the fat, or heterogeneous catalysis. To determine whether magnesium chloride was dissolved by dry butterfat, 100 ml. of freshly prepared butterfat was shaken at intervals for some hours at 60°C. with 20 g. of finely powdered magnesium chloride prepared by dehydrating in the vacuum oven at 100°C. followed by grinding. The magnesium chloride was then filtered from the fat, and the fat extracted with water. The chloride content of the water extract was estimated and compared with that of the extract of butterfat similarly treated throughout, but without the addition of magnesium chloride. There was no measurable difference in chloride content of the two extracts, indicating that less than 1 p.p.m. of magnesium chloride was in solution in the fat. Both fats were then carefully ashed, the proportions of ash being 1·2 mg. and 0·6 mg. per 100 ml. respectively. This difference is probably not significant. In any case it is clear that any degree of solution of the magnesium chloride in the fat or combination with it must be extremely small.

Surface catalysis might occur as a result of the formation of active centres through displacement of sodium and chloride ions in the crystal lattice. To test this, 0·1 per cent. of magnesium and other chlorides was added in solution to acid-washed sand and to sodium chloride, and the water then evaporated. The chlorides dispersed on

sand showed from 50 to 90 per cent. of the activity of the chlorides dispersed on sodium chloride. Account must be taken of the fact that the sand was relatively coarse (50 mesh against approximately 100 mesh for the salt) so it appears that any activation by sodium chloride is of relatively small magnitude.

Treatment of butterfat with alkali during preparation in a manner known to reduce the free fatty acid, slightly reduced the susceptibility of the fat to the oxidative action of magnesium chloride. This greater activity under acid conditions may be connected with the fact that the chlorides found to be oxidant tend to be acidic.

The effect of hydration and solution on the activity of magnesium chloride was studied by drying pure magnesium chloride in the vacuum oven at 100°C., then finely grinding and drying again. The degree of hydration was then found to correspond to 58 per cent. magnesium chloride. Equal lots of this magnesium chloride were then weighed in to the jars to be used for the oxidation experiment. Some were further treated in the vacuum oven until the magnesium chloride content reached 75 per cent. Another lot was exposed to moist air until the degree of hydration corresponded to the hexahydrate (46 per cent. magnesium chloride). This was accomplished without coalescence of the particles. To other lots water was added to give solutions of various strengths. The solids and solutions were dispersed in butterfat in the usual way. The oxidation values given in Table 9 suggest that

TABLE 9.—EFFECT OF VARIATION IN THE PROPORTION OF WATER PRESENT ON THE OXIDANT ACTIVITY OF MAGNESIUM CHLORIDE.

| Sample. | After Four Days at 40°C. | |
|---|---|---|
| | Thiocyanate Peroxide Value. | Lea Peroxide Value. |
| Control fat .. .. .. .. | A. 1·26, B. 1·30 → 1·28 | A. 0·60, B. 0·62 → 0·61 |
| Fat + *5 per cent. Mg $Cl_2$, dehydrated to 75 per cent. Mg $Cl_2$ .. .. .. | A. 3·16, B. 2·63 → 2·90 | A. 1·72, B. 1·51 → 1·62 |
| Fat + *5 per cent. Mg $Cl_2$, dehydrated to 58 per cent. Mg $Cl_2$ .. .. .. | A. 4·60, B. 4·57 → 4·59 | A. 1·84, B. 1·83 → 1·84 |
| Fat + *5 per cent. Mg $Cl_2$, dehydrated to 46 per cent. Mg $Cl_2$ .. .. .. | A. 4·63, B. 4·63 → 4·63 | A. 1·85, B. 1·74 → 1·80 |
| Fat + *5 per cent. Mg $Cl_2$, as 30 per cent. solution .. .. .. .. | A. 4·08, B. 4·00 → 4·04 | A. 1·63, B. 1·52 → 1·58 |
| Fat + *5 per cent. Mg $Cl_2$, as 20 per cent. solution .. .. .. .. | A. 3·47, B. 3·18 → 3·31 | A. 1·43, B. 1·42 → 1·43 |
| Fat + *5 per cent. Mg $Cl_2$, as 10 per cent. solution .. .. .. .. | A. 2·77, B. 2·70 → 2·74 | A. 1·24, B. 1·20 → 1·22 |

* 5 per cent. of the partly hydrated magnesium chloride containing 58 per cent. Mg $Cl_2$.

magnesium chloride is active in the presence of considerable concentration of water, but that continued dilution decreases the activity. The lessened activity when only 25 per cent. of water is present may be due to loss of hydrogen chloride in dehydrating the salt, or may indicate that some water is necessary to the catalysis.

The possibility that magnesium chloride affects the oxidation of fat by activating an enzyme was disproved by tests with fat heated under vacuum to 110°C. Oxidation of this heated fat was accelerated by magnesium chloride to the same extent as that of unheated fat.

It appears then that in the oxidation of fat, magnesium chloride functions directly as a heterogeneous catalyst, and that the presence of a certain proportion of water may be necessary for maximum activity.

### 7. The Oxidant Activity of Commercial Salt in Solution.

It was early observed that a solution of commercial salt emulsified in butter-fat accelerated oxidation. (Water alone—distilled from a Pyrex glass apparatus in use for over a year—had a consistent oxidant effect in all tests at 40°C.). This activity of commercial salt in solution was at first assumed to be also due to magnesium chloride, and experiments were undertaken to study the mode of action of magnesium chloride as a fat oxidant using aqueous solutions emulsified in fat. In particular the possibilities that the action was derived from the acidic nature of the salt, or perhaps from traces of free chlorine were studied. Anomalous results were obtained, and further experiments led to the finding that the activity of commercial salt in solution was not due to magnesium chloride. Typical results are given in Table 10.

TABLE 10.—THE EFFECT OF MAGNESIUM CHLORIDE ON THE OXIDANT ACTIVITY OF SALT IN SOLUTION.

| Sample. | | After Five Days at 40°C. | |
|---|---|---|---|
| | | Thiocyanate Peroxide Value. | Lea Peroxide Value. |
| Control fat | A. | 0·55 | 0·36 |
| | B. | 0·57 | 0·34 |
| | Mean | 0·56 | 0·35 |
| Fat + 16 per cent. dist. water | A. | 0·89 | 0·50 |
| | B. | 0·99 | 0·51 |
| | Mean | 0·94 | 0·51 |
| Fat + 16 per cent. dist. water + 5 per cent. A.R. NaCl | A. | 1·39 | 0·67 |
| | B. | 1·37 | 0·70 |
| | Mean | 1·38 | 0·69 |
| Fat + 16 per cent. dist. water + 5 per cent. A.R. NaCl containing 0·1 per cent. $MgCl_2$ | A. | 1·35 | 0·68 |
| | B. | 1·24 | 0·63 |
| | Mean | 1·30 | 0·66 |
| Fat + 16 per cent. dist. water + 5 per cent. commercial salt | A. | 1·78 | 0·89 |
| | B. | 1·71 | 0·81 |
| | Mean | 1·75 | 0·85 |
| Fat + 16 per cent. dist. water + 5 per cent. commercial salt heated to 700°C. | A. | 1·70 | 0·84 |
| | B. | 1·66 | 0·77 |
| | Mean | 1·68 | 0·81 |
| Control fat | A. | 0·97 | 0·51 |
| | B. | 0·93 | 0·50 |
| | Mean | 0·95 | 0·51 |
| Fat + 16 per cent. dist. water | A. | 1·50 | 0·69 |
| | B. | 1·52 | 0·71 |
| | Mean | 1·51 | 0·70 |
| Fat + 16 per cent. dist. water + 5 per cent. A.R. NaCl | A. | 1·57 | 0·75 |
| | B. | 1·66 | 0·83 |
| | Mean | 1·62 | 0·79 |
| Fat + 16 per cent. dist. water + 5 per cent. A.R. NaCl containing 0·1 per cent. $MgCl_2$ | A. | 1·67 | 0·79 |
| | B. | 1·50 | 0·73 |
| | Mean | 1·59 | 0·76 |
| Fat + 16 per cent. dist. water + 5 per cent. A.R. NaCl containing 1·0 per cent. $MgCl_2$ | A. | 1·61 | 0·72 |
| | B. | 1·75 | 0·81 |
| | Mean | 1·68 | 0·77 |
| Fat + 16 per cent. dist. water + 5 per cent. A.R. NaCl containing 5·0 per cent. $MgCl_2$ | A. | 1·75 | 0·80 |
| | B. | 1·73 | 0·83 |
| | Mean | 1·74 | 0·82 |
| Fat + 16 per cent. dist. water + 5 per cent. A.R. NaCl containing 20 per cent. $MgCl_2$ | A. | 1·86 | 0·88 |
| | B. | 1·89 | 0·88 |
| | Mean | 1·88 | 0·88 |

The oxidant effect of commercial salt in aqueous solution dispersed in fat must be due to impurities other than magnesium chloride. Traces of heavy metals may be active under these conditions and Lea (1934) has shown that when buffer solutions at pH 7·0 are in contact with fat, 0·1 p.p.m. of copper in the solution will accelerate oxidation. The commercial salt solution in the experiment reported in Table 10 would have contained about 0·2 p.p.m. of copper. Lea found that the effect of such small traces of metal was overcome by the presence of milk or protein. In butter, therefore, where the concentration of salt is much lower and the corresponding copper increment would be 0·010 to 0·015 p.p.m., it seems unlikely that metallic contamination can explain the marked acceleration of oxidation by salt. Wiley (1939) showed that this acceleration was apparent even with sweet cream butters (serum pH 6·6), but was very obvious in acid butters (serum pH 5·0). A butter storage experiment confirmed these findings and showed that heating to 700°C. did not diminish the oxidant activity of salt under these conditions. Whether the oxidant activity of salt in butter is due to metallic impurities, to a salt effect in acid catalysis, or to some other cause, remains to be elucidated.

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# Measurement of the Gas Content of Concentrated Butter and Other Fat Products.

*By G. Loftus Hills, B.Agr.Sc.,* and J. Conochie, B.Sc.(Agric.).**

*Summary.*

For routine control purposes, it was necessary to develop a method for the determination of gas in concentrated butter. A modification and refinement of the method for determination of air in butter of Rahn and Mohr is described. The method should be applicable to other fat products. Provision is made for the determination of free gas and gas in solution. The relation of the modifications to the measurement of gas in butter is discussed. Diffusion of gases through plastic fats occurs with significant rapidity.

## 1. Introduction.

Concentrated butter for use under tropical conditions and where refrigeration facilities are not available is made by adding about 3·5 per cent. of fully hydrogenated peanut oil to melted butter-fat, incorporating 2 per cent. of finely ground salt and 4 per cent. of milk powder, de-aerating and then cooling rapidly to give a smooth texture. This product largely depends for its resistance to oxidative deterioration on low oxygen content. A method was therefore required for the routine measurement of the gas content of the product.

In the method of Rahn and Mohr (1924), later slightly modified by Guthrie (1930), for the measurement of the air content of butter, a metal cone (C) filled with butter is introduced in gas free water into an apparatus of the general type shown in Fig. 1. Immersion of the apparatus in a water bath at 40°C. melts the butter. Gas present rises to the top of the inner glass vessel (V). Application of change of pressure to the system through the pipette (P) results in change of volume due solely to expansion or contraction of the released gas. From measured pressure and corresponding volume changes, the volume of the gas is calculated and related to the weight of butter in the cone.

Although the butter is held under reduced pressure during melting, this method does not take account of the time factor in the removal of gases from solution in fat. In butter the bulk of the gas is probably present not in solution, but as dispersed gas bubbles. For concentrated butter which has a lower total gas content and in which the gas is normally all in solution in the fat, or present as core air in the spray-dried milk particles, it was necessary to modify the method.

## 2. Modification of the Rahn and Mohr Method.

The apparatus was altered in two minor ways. Firstly, the inner glass vessel (V) was made larger, so that the metal cone fitted into it. With this modification a rubber connexion between cone and glass was unnecessary, and no fat escaped into the outer container. Secondly, the curved tube fitted inside the top of the glass cap by Guthrie could then be omitted, so that should any small bubble of gas be trapped by mischance in the apparatus during assembly it could, when expanded under vacuum, rise freely to the surface and escape.

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With the low gas content of concentrated butter, any air bubbles adhering to the fat surfaces at each end of the metal cone would introduce considerable error. These were removed by scraping the ends with a spatula under gas-free water during the assembling of the apparatus. This at the same time reduced possible error from diffusion of gas into the end surfaces of the cone of butter.

For the higher melting concentrated butter, it was necessary to use a higher water bath temperature, 45°C., and to extend the time of melting.

To remove gas from solution in the melted fat in the assembled apparatus, it was necessary to reduce the pressure to a low value; this was limited by the vapour pressure of the water (72 mm. mercury at 45°C.) and the necessity for allowing a margin of safety to avoid boiling the water. A pressure of 80 mm. was adopted for routine determinations. The relationship between time of application of this vacuum and removal of gas is shown by the figures in Table 1.

Table 1.—The Effect of Time of Extraction on the Removal of Gas from Concentrated Butter.

| Sample A. | | Sample B. | | Sample C. | | Sample D. | |
|---|---|---|---|---|---|---|---|
| Time. | Volume at N.T.P./100g. | Time. | Volume at N.T.P./100g. | Time. | Volume at N.T.P./100g. | Time. | Volume at N.T.P./100g. |
| min. | ml. | min. | ml. | min. | ml. | min. | ml. |
| 10 | 0·48 | 10 | 0·98 | 0 | 0·01 | 0 | 0·06 |
| 20 | 0·80 | 21 | 1·49 | 10 | 0·25 | 10 | 0·82 |
| 30 | 0·89 | 33 | 1·86 | 60 | 0·56 | 85 | 2·00 |
| 40 | 0·94 | 47 | 2·05 | 90 | 0·62 | .. | .. |
| 50 | 0·95 | 72 | 2·18 | .. | .. | .. | .. |
| 110 | 0·98 | 95 | 2·21 | .. | .. | .. | .. |
| 230 | 1·04 | .. | .. | .. | .. | .. | .. |

The volume after ten minutes extraction was found to be between 40 per cent. and 50 per cent. of the total volume, and for routine process control tests where rapid determination was necessary a ten-minute extraction period was adopted. The percentage of gas so observed was called the Gas Index.

In calculating the Gas Index a correction was made for the hydrostatic head on the gas bubble, and the results were expressed as ml. of dry gas at N.T.P. per 100 g. of fat. The method of calculation assumes that the bubble is saturated with water vapour. Since the bubble is surrounded by fat this assumption might be questioned, but calculations of values from a range of volume and pressure measurements give results agreeing so closely that the correction for water vapour pressure must be valid.

### 3. Application of the Modified Method.

The apparatus is shown in Fig. 1. The metal cone, for which suitable dimensions are height 44 mm., small diameter 22 mm., large diameter 28 mm., holds approximately 20 g. The cone must be smoothly finished to avoid air pockets.

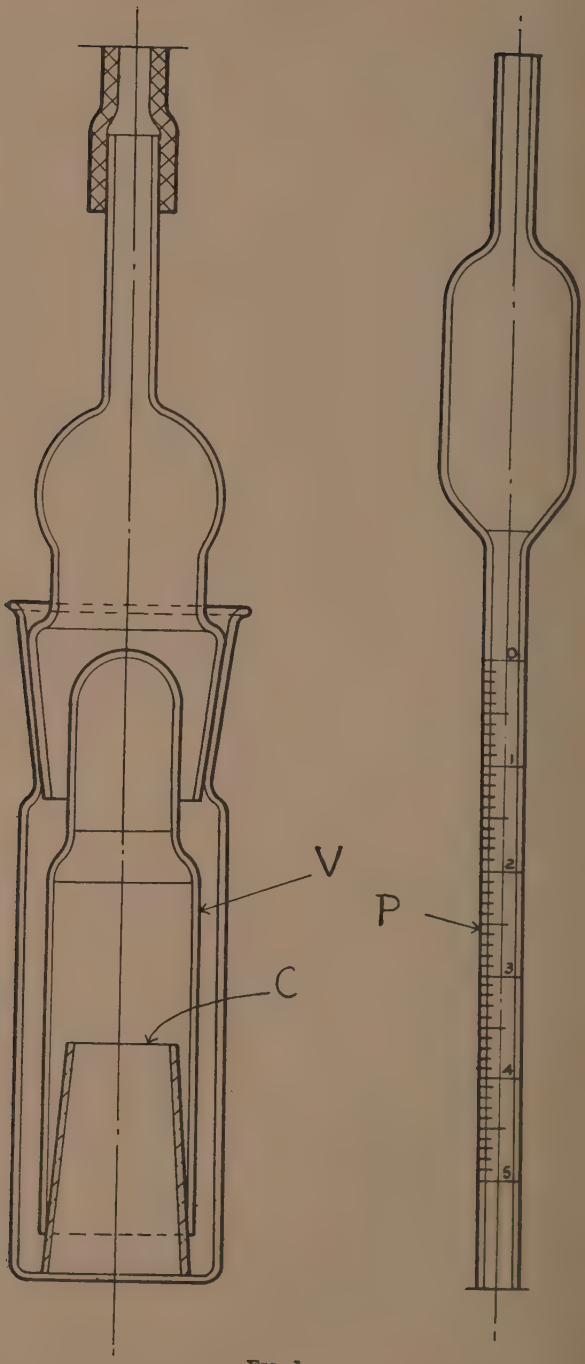


Fig. 1.

Sample the product immediately it is opened. With 1-lb. tins it is often convenient to open both ends and push the product from the tin. Push a metal cone of known weight into the product until ½ inch of product emerges from the end. Cut out the metal cone, trim the ends, wipe clean, and weigh.

For each determination, freshly boil water and cool it under vacuum. Pour the water into a suitable flat-bottomed container to a depth of about 3 inches, taking care to dissolve a minimum of air. Coat the male part of the ground glass joint with heavy stop-cock grease. If the glass parts of the apparatus are wet, rinse them with gas-free water and then immerse them in the bath carefully so that they contain no air bubbles. Good illumination of the assembling bath is necessary for the detection of air bubbles. Bring the metal cone containing the butter or other product under the water, remove adhering air bubbles from the metal, and scrape the ends free from air bubbles, removing a layer of 1/16 inch to ⅛ inch of product in the process. Place the removed product in an aluminium dish of known weight, and later dry by heating carefully over a flame, cool, weigh, and calculate the weight of product remaining in the cone.

Assemble the apparatus under water, remove from the bath, and attach the pipette, taking care that the ends of the two glass tubes touch to minimize movement of the connecting pressure tubing on change of pressure. Immerse the assembled apparatus in a well-stirred water-bath maintained at 45 ± 0·1°C. A rubber stopper placed between the bottom of the water-bath and the apparatus enables slight pressure to be maintained on the ground glass joint when the apparatus is clamped in position. The level of water in the bath should be just above the rubber tubing. To the top of the pipette, attach pressure tubing leading to a mercury manometer and to a 2-l. surge vessel opening through stopcocks to a vacuum system and to the atmosphere.

Expansion on warming will bring the water level inside the apparatus up to the scale of the pipette. After some time the butter will rise out of the cone to the top of the inner glass vessel. Allow at least a further five minutes for complete melting, the total time for concentrated butter being about twenty minutes. The rate at which determinations are made can be increased by having several sets of apparatus in the water-bath and connecting each in turn to the vacuum system.

When melting is completed, make a measurement of the free gas in the product by rapidly reducing pressure to 300 mm. Read the pipette as soon as the manometer is approximately steady, then open the system to atmospheric pressure and again record the pipette reading. By taking the volume reading at atmospheric pressure after, rather than before, the pressure change, error due to settling in of the ground glass joint or the rubber tubing is avoided. From the volume change observed, deduct a blank value for the apparatus pre-determined by applying various pressure changes when only boiled water is present. This will usually not exceed 0·01 ml. Measurement of the free gas must be made rapidly to avoid extraction of gas from solution. Calculate the percentage of free gas in the same way as is shown below for the Gas Index.

Reduce the pressure to 80 mm., calculating the appropriate reading for an open type of manometer from the barometric pressure. Maintain this pressure within $\pm$ 0·2 mm. during the extraction time of exactly ten minutes. Tap the walls of the pipette to remove any gas bubbles which form on the inner walls. At the end of the extraction period increase the pressure somewhat before reading the pipette. If the volume of the gas is high this increase will be necessary to bring the meniscus to the pipette scale, but it is in any case desirable to increase the pressure to 100 mm. since corrections are less critical than at 80 mm. Take the volume and pressure readings and then open the system to atmosphere. Allow half a minute for draining and again read the volume. Check measurements may be made by again immediately reducing the pressure. These tend to be progressively lower because the gas is re-absorbed while at atmospheric pressure.

Calculate the free gas and the Gas Index as follows:—

$$V_1 = \frac{V_m}{\frac{A_1 - p_1 + p_2}{A_2 - p_1 + p_3} - 1}$$

Percentage gas at N.T.P. expressed as ml./100 g.

$$V_1 \times \frac{273}{273 + t_1} \times \frac{A_1 - p_1 + p_2}{760} \times \frac{100}{W}.$$

where—

$V_m$ = Volume change measured in pipette in ml. less blank value for the apparatus for the same change in pressure.

$A_1$ = Barometric reading in mm. mercury, not corrected for temperature.

$A_2$ = Pressure on water surface in pipette when measuring expanded volume, in mm. mercury.

$p_1$ = Vapour pressure of water at $t_1$°C.

$p_2$ = Equivalent in mm. mercury of the height of water above the gas bubble, when the water surface is at atmospheric pressure.

$p_3$ = Equivalent in mm. mercury of the height of water above the gas bubble, when the water surface is at pressure $A_2$.

$W$ = Weight of butter initially taken less weight of butter removed in scraping ends.

$t_1$ = Temperature of water bath in degrees C.

## 4. Discussion.

The method has proved very useful in practice, but two limitations have been borne in mind. With low values the residual gas in the product at equilibrium becomes significant. When an extraction pressure of 80 mm. is used at 45°C., the theoretical equilibrium gas content of butter-fat is 0·1 per cent. The second limitation concerns

the composition of the gas. With butter-fat which has been in equilibrium with air during manufacture, approximately 30 per cent. of the gas present will be oxygen. This may be calculated from the figures for the solubility of gases in butter-fat given by Schaffer and Haller (1943). At times this proportion has been greatly altered by the presence of carbon dioxide derived from sodium carbonate used to treat the salt added to concentrated butter. It is then necessary to carry out the determination using caustic soda solution instead of water, and half-normal is the minimum strength found to be satisfactory. An alternative form of the apparatus has also been devised in which the ground glass joint was placed at the bottom, a side arm fitted for attachment of the pipette, and an extension at the top fitted with a tap and cup through which strong alkali could be admitted after extraction of the gas. Values obtained with this apparatus agree well with those obtained with the usual apparatus and half-normal caustic soda.

## 5. Gas Determination in Butter.

The significance of the modifications here described for the determination of gas in butter will depend on the proportion which is in solution. Rahn and Mohr, and Guthrie, do not discuss this matter in describing their method, but seem to assume that the gas is free. Mohr and Eysank (1943) heat butter under glycerol to liberate gas; this should remove part of the gas in solution. Mennicke (1942) uses a centrifugal method of which details are not given in the abstract available.

Schaffer and Haller give the solubility of air in butter-fat at 40°C. as 10·1 ml. per 100 ml. At room temperature the glycerides of butter-fat are by no means all solid. Bailey, Todd, Singleton, and Oliver (1944) found that with fats in their plastic temperature range, about 80 per cent. of the glycerides were in the liquid state. Bailey and Kramer (1944) give results from their dilatometric study of fats which indicate that 50 per cent. of butter-fat is liquid at 20°C. Even if the solubility of air in solid triglycerides be neglected, it is clear that butter in equilibrium with air, a state to be expected from the conditions of manufacture, should contain about 4 per cent. of gas in solution.

Study of a butter which had been worked under vacuum showed 1·1 per cent. of free gas and 2·4 per cent. total gas after two hours' extraction. A normal butter showed 5·4 per cent. free gas and 7·6 per cent. total gas after two hours' extraction. These results suggest that although the gas in solution in butter may be less than expected from theoretical considerations, it should not be neglected in measuring the gas content of butter.

## 6. Diffusion of Gas through Fat.

Samples of concentrated butter exposed to the air overnight were found to increase appreciably in Gas Index. Absorption of gas was also evident if the cones of butter were exposed to the air for even short periods (of the order of one hour) after the initial trimming, and

an inadequate layer of butter then removed under water. This appreciable diffusion of gas through the fat supported earlier observations in this laboratory in which reduced methylene blue was used to indicate diffusion of oxygen through butter-fat.

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* Not seen in the original.

# The Keeping Quality of Australian Milk Powders.

*By C. C. Thiel, B.Sc.(Agric.), Ph.D.,* and E. G. Pont, M.Sc.Agr.**

*Summary.*

A preliminary survey of the relative keeping qualities of several Australian spray-dried and roller-dried, skim and whole milk powders has been made. Gas-packing did not materially improve the storage life of skim milk powders but greatly increased the life of whole milk powders. In commercial practice the advantages of gas packing were largely nullified by the presence of leaking tins. Skim milk powders when reconstituted after storage with fresh flavoured butterfat were no better in flavour than reconstituted gas-packed whole milk powders stored under identical temperature conditions. Variations in the keeping quality of powders made at different times by any one factory were considerable, and great differences in the keeping quality of whole milk spray-dried powders from different factories were noticed. The roller-dried whole milk powders examined were inferior in flavour to the better quality spray-dried whole milk powders. The influence of storage temperatures (15°, 30°, and 37° C.) was not marked either with the skim or whole milk powders. No correlation between bacterial counts and initial quality or condition after storage was observed.

## 1. Introduction.

As no published information is available on the relative keeping qualities of different types of Australian milk powder, a preliminary survey using Victorian products was undertaken. The powders were compared as normally packed commercially: that is the skim milk powders in friction-lid tins and whole milk powders gas-packed in tins. At the same time the opportunity was taken of comparing skim milk powders packed in air and gas-packed, and whole milk powders packed in air, gas-packed to 3·5 per cent. oxygen in the headspace gas, and double gas-packed to 0·6 per cent. oxygen in the headspace gas. As different types of powder have different packing densities, the less dense powders had more oxygen available to them at a given oxygen tension than more dense powders. Again, this is in accordance with normal commercial conditions. Powders were obtained from five factories in all, but as Factories C and E had no gas-packing equipment, bulk samples of powder from these factories were sent to Factory A for tinning and gassing. The tins in which the powders were packed were of about 600 ml. volume.

## 2. General Plan of the Experiment.

### (i) *Samples Taken.*

As it was impossible to obtain various types of powder made from one bulk of milk from any one factory, three sets of samples were taken at weekly intervals from each factory, some of which produced only one type of powder and others more than one. In this way it was hoped to minimize discrepancies due to comparing different types of

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powders made from separate bulk samples of milk, and also to obtain some information on the extent of daily variation in the powders produced by any particular factory. The three sets of samples taken at weekly intervals have been called Series 1, 2, and 3. Details are given in Table 1 of the samples taken from each factory for each series, the manner in which they were packed and the temperatures at which they were stored. The figures quoted for oxygen content of the headspace gas were obtained after the tins had been kept for ten days. It will be noticed that the figures for Series 2 powders vary considerably from factory to factory, and also within the samples from each factory.

TABLE 1.—TYPES OF MILK POWDER, METHODS OF PACKING, AND STORAGE TEMPERATURES.

| Factory. | Type of Powder. | Type of Packing. | Storage Temperatures. |
|---|---|---|---|
| | | | Deg. C. |
| A. | Skim spray .. .. | Friction lid .. .. .. | 15, 30, 37 |
| | | Sealed tin with air .. .. | 37 |
| | | Gas-packed 3·5 per cent. $O_2$ .. | 37 |
| B. | Skim spray .. .. | Friction lid .. .. .. | 15, 30, 37 |
| | | Sealed tin with air .. .. | 37 |
| | | Gas-packed 3·5 per cent. $O_2$ .. | 37 |
| B. | Skim roller .. .. | Friction lid .. .. .. | 15, 30, 37 |
| | | Sealed tin with air .. .. | 37 |
| | | Gas-packed 1·5 per cent. $O_2$ .. | 37 |
| C. | Skim roller (gas-packed factory A) | Friction lid .. .. .. | 15, 30, 37 |
| | | Sealed tin with air .. .. | 37 |
| | | Gas-packed 1·5 per cent. $O_2$ .. | 37 |
| A. | Whole milk spray .. | Sealed tin with air .. .. | 37 |
| | | Gas-packed 3·5 per cent. $O_2$ (3·4 per cent.)* | 15, 30, 37 |
| | | Gas-packed 0·6 per cent. $O_2$ .. | 37 |
| D. | Whole milk spray .. | Sealed tin with air .. .. | 37 |
| | | Gas-packed 3·5 per cent. $O_2$ (4–6 per cent.)* | 15, 30, 37 |
| | | Gas-packed 0·6 per cent. $O_2$ .. | 37 |
| B. | Whole milk spray .. | Sealed tin with air .. .. | 37 |
| | | Gas-packed 3·5 per cent. $O_2$ (6–8·5 per cent.)* | 15, 30, 37 |
| | | Gas-packed 0·6 per cent. $O_2$ .. | 37 |
| E. | Whole milk roller (Series 1 only, gas-packed, factory A) | Sealed tin with air .. .. | 37 |
| | | Gas-packed 1·3 per cent. $O_2$ (2·4 per cent.)* | 15, 30, 37 |
| | | Gas-packed 0·3 per cent. $O_2$ .. | 37 |
| B. | Whole milk roller .. | Sealed tin with air .. .. | 37 |
| | | Gas-packed 1·3 per cent. $O_2$ (6–7·5 per cent.)* | 15, 30, 37 |
| | | Gas-packed 0·3 per cent. $O_2$ .. | 37 |

* Figures in brackets refer to the percentage oxygen in the headspace gas of Series 2 powders which were not regassed in the laboratory.

(ii) *Gas-Packing.*

It was originally intended to store the gas-packed samples as received from the factories, but the variation in headspace oxygen from factory to factory was so great that it was decided to repack to a

uniform oxygen tension in the headspace for similar powders from different factories. This was done for Series 1 and 3 powders but Series 2 powders were not repacked (leaking tins were rejected).

As spray-dried powders contain core air and hence "desorb" on standing, a single gassing will not reduce the final oxygen concentration to a value as low as when roller-dried powders are treated in the same manner. For this reason headspace oxygen concentrations which could be attained by efficient factory apparatus were selected; these were 3·5 and 0·6 per cent. for single and double gas-packed spray-dried powder, and 1·5 and 0·3 per cent. for roller-dried powder. Because of desorption it was not easy to obtain exactly the required oxygen concentration in the headspace gas of tins of spray-dried milk powder, but close approximation to the required values was obtained in the following manner. The powders were regassed in the laboratory using a high vacuum (3 mm. mercury residual pressure) and nitrogen containing 0·2 per cent. oxygen. After ten days the powders were once more regassed using nitrogen containing 3·2 per cent. oxygen, when 3·5 per cent. final oxygen concentration was required, and nitrogen containing 0·2 oxygen to obtain final values of 0·6 per cent. With the roller-dried powders a single regassing on arrival from the factory was sufficient, using nitrogen containing 0·2 per cent. less oxygen than the final value required.

After the final gassing the tins of milk powder were kept for a further ten days at room temperature, and the oxygen concentrations in the headspace gas were then determined. About 20 ml. of gas was abstracted for the analyses but as all tins were treated in a similar manner no correction has been applied for this reduction of the amount of oxygen available to the milk powder.

(iii) *Storage Temperatures and Times of Grading.*

Samples stores at 37°C. were examined after 4, 10, 14, 24, and 40 weeks. At 30° they were examined after 6, 14, 22, 39, and 61 weeks, and at 15° after 26, 39, and 61 weeks.

### 3. Methods of Analysis, Grading, and Testing Tins for Leaks.

(i) *Moisture.*

A 1 g. sample was heated in a $2\frac{3}{4}$ in. by $\frac{1}{2}$ in. aluminium dish for six hours at 98°C. in a steam-jacketed vacuum oven (A.O.A.C., 1935).

(ii) *Copper and Iron.*

A 5 g. sample was ashed by heating gently over a bunsen burner followed by heating in a furnace not above 475°C. After cooling, 0·5 ml. redistilled concentrated sulphuric acid was added and the dish reheated over a burner to give a white ash. Overheating at this stage produces insoluble calcium sulphate. The ash was then taken up in 5 ml. of boiling 6N hydrochloric acid to convert pyrophosphate to the ortho form and the volume made to 40 ml. Iron was determined in an aliquot of this solution using thiocyanate, and copper by the dithiocarbamate method of Sylvester and Lampitt (1935).

(iii) *Oxygen and Carbon Dioxide.*

A conventional Haldane gas analysis apparatus was used. In order to seal the tins again after withdrawing a sample for analysis, an electrically heated soldering bit enclosed in a cell clamped to the tin was used. The cell and tube connecting it to the gas analysis apparatus were evacuated to 0·01 mm. residual pressure before the tin was pierced.

(iv) *Grading.*

An important aim of the experiment was to compare on a flavour basis reconstituted gas-packed whole milk powders with skim milk powders packed in friction-lid tins and reconstituted after storage with fresh flavoured butterfat. Butterfat was obtained as required by slowly melting best quality butter and decanting the clear butterfat. To reconstitute skim milk powders, 34 g. of powder, 15 ml. of butterfat, and 350 ml. of water at 60° were shaken in a flask for one minute using a mechanical shaker, and the mixture then passed through a hand-homogenizer. Whole milk powders were reconstituted by shaking 50 g. of powder in 400 ml. water at 60°C. for one minute. All samples were then cooled for one hour in tap water before grading.

The system of grading used by Lea, Moran, and Smith (1943) was adopted for this work. The grades were defined as follows:—

0 = as good as best obtainable fresh spray-dried whole milk powder.

1 = a suspicion of "off" flavour.

2 = a definite "off" flavour.

3 = unpalatable.

4 = very unpalatable.

This system is slightly different from the original in that 0 grade is defined on the basis of the best quality whole milk powder available, whereas Lea used control samples stored at —20°C. and defined his 0 grade as being as good as the control. The powders were graded by five members of the staff of the C.S.I.R. Dairy Section and one or (more often) two of the senior graders of the Commonwealth Department of Commerce. The scores of individual graders were averaged and the 0 to 4 grades further divided by the use of + and — signs.

Roller-dried powders, being less soluble than spray-dried powders, are less acceptable to most people as liquid milk because of the physical sensation on the palate. As far as possible samples were not degraded because of their physical nature, but scored only for flavour. Thus scorched and heated flavours whether present in the fresh powder or developed during storage were taken account of, but the unpleasant feel of undissolved protein was neglected.

(v) *Testing Tins for Leaks.*

As soon as received from the factories all tins with reamed tops were tested for leaks. This was done by placing them overnight under a constant air pressure of 5 lb./sq. inch in a closed vessel and next morning releasing the pressure and as quickly as possible examining each tin under water for leaks. Leaking tins were sealed with Wood's metal by rotating the faulty seam in a layer of the molten metal covered with lactic acid as a flux.

## 4. Results and Discussion.

The extensive data obtained by the examination of some 700 tins of powder have not been given in detail in the following section. Only illustrative data are reported.

(i) *Packing Densities and Moisture, Copper, and Iron Contents of Milk Powder.*

These data for the various lots of powder from each factory are given in Table 2. It will be noticed that, in general, analytical figures are more constant for fullcream than for skim milk powders for the three series taken at weekly intervals.

Table 2.—Packing Density and Moisture, Copper, and Iron Contents of Milk Powders.

| Code.* | Description. | Gas-packed by Factory. | Headspace (ml. per g. of Powder). | Moisture. | Cu. | Fe. |
|---|---|---|---|---|---|---|
| | | | | % | µg/g. | µg/g. |
| ASS1 .. | Spray skim .. | A. | 0·67 | 4·0 | 3·4 | 3·4 |
| ASS2 .. | ,, ,, .. | A. | 0·66 | 4·6 | 2·0 | 2·4 |
| ASS3 .. | ,, ,, .. | A. | 0·70 | 4·2 | 2·3 | 2·4 |
| BSS1 .. | ,, ,, .. | B. | 0·80 | 4·4 | 2·3 | 2·6 |
| BSS2 .. | ,, ,, .. | B. | 0·65 | 3·6 | 2·2 | .. |
| BSS3 .. | ,, ,, .. | B. | 0·89 | 4·1 | 2·4 | 2·0 |
| BSR1 .. | Roller skim .. | B. | 1·60 | 3·2 | 3·9 | 9·9 |
| BSR2 .. | ,, ,, .. | B. | 2·60 | 1·5 | 2·2 | 28·0 |
| BSR3 .. | ,, ,, .. | B. | 2·40 | 2·7 | 2·5 | 13·1 |
| CSR1 .. | ,, ,, .. | A. | 1·65 | 2·5 | 3·5 | 8·6 |
| CSR2 .. | ,, ,, .. | A. | 1·78 | 2·5 | 4·1 | 11·6 |
| CSR3 .. | ,, ,, .. | A. | 1·55 | 3·9 | 3·7 | 10·2 |
| AFS1 .. | Spray whole milk .. | A. | 0·90 | 3·0 | 0·8 | 1·4 |
| AFS2 .. | ,, ,, ,, .. | A. | 0·94 | 2·8 | 1·3 | 2·4 |
| AFS3 .. | ,, ,, ,, .. | A. | 0·94 | 2·8 | 1·4 | 2·5 |
| DFS1 .. | ,, ,, ,, .. | D. | 1·02 | 2·7 | 0·36 | 2·0 |
| DFS2 .. | ,, ,, ,, .. | D. | 1·02 | 2·2 | 0·30 | 3·1 |
| DFS3 .. | ,, ,, ,, .. | D. | 1·02 | 2·6 | 0·39 | 2·9 |
| BFS1 .. | ,, ,, ,, .. | B. | 0·97 | 2·0 | 2·8 | 2·5 |
| BFS2 .. | ,, ,, ,, .. | B. | 0·95 | 2·6 | 2·7 | 4·0 |
| BFS3 .. | ,, ,, ,, .. | B. | 1·00 | 2·1 | 2·3 | 3·6 |
| EFR1 .. | Roller whole milk .. | A. | 1·77 | 1·9 | 0·46 | 13·5 |
| BFR1 .. | ,, ,, ,, .. | B. | .. | 3·2 | 1·9 | 11·6 |
| BFR2 .. | ,, ,, ,, .. | B. | 1·70 | 3·2 | 1·1 | 10·7 |
| BFR3 .. | ,, ,, ,, .. | B. | 2·00 | 2·7 | 1·6 | 14·5 |

* 1st code letter = Factory.
2nd code letter = Skim (S) or whole milk (F).
3rd code letter = Spray (S) or roller (R).
Number at end = Series 1–3 samples taken at weekly intervals.

The wide variation in packing densities of skim milk powders from any one factory taken at weekly intervals was not foreseen. At the time the samples were taken, manufacturers were not regularly packing skim milk powder in small friction-lid tins, and they were asked to fill the tins completely, jolting each tin a few times to pack the powder to an even density. As a result, it was found that the weight of powder was constant in the tins of each series, but this weight varied

considerably for the different lots of powder taken at weekly intervals. This indicates that some powders were much more fluffy than others, but under normal commercial conditions of packing a constant weight would have been forced into each tin.

(ii) *Air-tightness of Tins.*

It is obvious that unless tins are gas-tight it is a waste of effort to gas-pack milk powder in them. It was therefore disappointing to find that 41 per cent. of the tins gas-packed by Factory A were found to leak by the test applied, 34 per cent. of those packed by Factory B leaked, and 21 per cent. of those packed by Factory D.

The general practice in Victoria is to obtain tins from canister makers ready made, but with one end open. At the factories, the tins are filled and the remaining end reamed on. It was therefore interesting to note that, with rare exceptions, leaks were only detected in the seam made at the milk plant, and. again with rare exceptions, all leaks occurred at the junction of this seam and the side seam.

It was possible that the external pressure of 8 lb./sq. inch applied overnight to the tins damaged the seams sufficiently to cause them to leak. Although the seams made at the milk plants were less able to withstand this pressure than the seams made by the canister manufacturers, all the seams might have remained tight under normal handling condition. To obtain further information on the frequency of leaking tins, a few 12 oz. tins gas packed by Factories A and D were obtained from retail shops and the headspace gas analysed for oxygen concentration. No external pressure was applied to these tins. Four of the five tins from Factory A showed more than 18 per cent. oxygen in the headspace gas and one of the five from Factory D. An internal pressure of 1 lb./sq. inch was sufficient to show leaks at the usual point when the tins were held under water. At a later date five tins each containing 12 lb. of milk powder were obtained from Factory B. These were hand-soldered rectangular containers and had been gas-packed. All contained more than 20 per cent. oxygen in the headspace gas. The fact that a high proportion of tins which had suffered only normal commercial handling were found to be leaking confirmed the results obtained using the air pressure method of detecting leaking tins, and it may be concluded that insufficient attention is given by manufacturers to the adjustment of reaming machines.

The rate at which the oxygen content of the headspace gas increases if the tins are imperfectly sealed, was demonstrated by the following experiment. Five tins of roller-dried powder selected had barely detectable leaks when examined by the external air pressure method. They were gas-packed to contain about 1·3 per cent. oxygen in the headspace gas and then stored at room temperature close to a maximum and minimum thermometer. After eight days, the headspace gas of one tin contained 3·9 per cent. oxygen, after 22 days another tin showed 5·1 per cent. oxygen, and after four months the remaining three

tins showed 15–19 per cent. oxygen. The average difference between maximum and minimum temperature readings for this period was 14·4°F.

Gas-packing of whole milk powder for retail trade is carried out by Australian manufacturers on their own initiative to increase the keeping quality of their product. As will be shown later in this paper, gas-packing greatly increases the life of whole milk powder and therefore, if properly carried out, is commercially profitable. If, however, as has been shown to occur in Victoria, a high percentage of the tins leak, then some tins of powder will retain their good quality, while others will deteriorate. Such unevenness in quality of a product will certainly effect the consumers' attitude to it.

(iii) *Keeping Quality of Skim Milk Powders.*

(*a*) *Effect of temperature of storage.*—After storage for 26 weeks at 15°, 30°, and 37°C., skim milk powders showed marked variation in quality. As may be seen from Table 3, the spray-dried samples stored at 15° had shown little change, while the roller-dried samples had altered by about 1 grade unit. At 30° the changes were of the order of 0·5 and 1·5 units for spray-dried and roller-dried samples, and at 37° the differences were both 2 units for the two types of powder. However, after storage for 39 and 61 weeks, there were no significant differences between samples stored at 15°, 30°, and 37°. It is evident from Table 3 that at 37° little or no additional deterioration occurred after 26 weeks, whereas at 15° and 30° a relatively steady grading score was attained at some time between 26 and 39 weeks. Earlier examination of the samples stored at 37° indicated that the change from rapid deterioration occurred between 14 and 24 weeks.

(*b*) *Comparison of spray-dried and roller-dried skim milk powder.*—The roller-dried samples were less acceptable than the spray-dried samples as reconstituted whole milk from the point of view of flavour at the time they were placed in store. In addition, the roller-dried powders deteriorated more rapidly than the spray-dried powders, scorched and gluey flavours tending to develop earlier. At the end of the experiment, however, there was little to choose between the two types.

(*c*) *Variation of samples from individual factories.*—Variation in the quality of powders from individual factories were not striking, except in the case of Factory A. Tallowy flavours developed in samples ASS1 which accordingly graded badly, whereas samples ASS2 and ASS3 were consistently better than all the other skim milk samples. The development of talloviness in sample ASS1 was no doubt due to its high fat content (2·58 per cent.) compared with samples ASS2 and ASS3 which contained 0·78 and 0·89 per cent. fat respectively.

(*d*) *Effect of gas-packing on samples stored at* 37°*C.*—Gas-packing had no more than a slight retarding effect on the development of off-flavour in skim milk powders stored at 37°. At all examinations six gas-packed samples graded on the average ½ grade unit worse than the corresponding air-packed samples, 33 the same, and 16 on the

TABLE 3.—EFFECT OF STORAGE TEMPERATURE ON KEEPING QUALITY OF SPRAY-DRIED AND ROLLER-DRIED SKIM MILK POWDERS PACKED IN FRICTION-LID TINS.

| Sample. | Grade before Storage. | Moisture. | Cu. | Fe. | 26 Weeks. | | | 39 Weeks. | | | 61 Weeks. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 15.° | 30.° | 37.° | 15.° | 30.° | 37.° | 15.° | 30.° |
| | | % | μg/g. | μg/g. | | | | | | | | |
| ASS1 .. .. | G 1 | 4·0 | 3·4 | 3·4 | S 1 | OS 2 | 4 | SO 3 | SO 4 | −2 | SG 3 | OSG 4 |
| ASS2 .. .. | G −1 | 4·6 | 2·0 | 2·4 | S −1 | S 1 | 3 | S 2 | S −2 | −2 | SG +1 | SG +1 |
| ASS3 .. .. | G −1 | 4·2 | 2·3 | 2·4 | S −1 | S 1 | +1 | G 3 | S 2 | +2 | SG +1 | SG 2 |
| BSS1 .. .. | G −1 | 4·4 | 2·3 | 2·6 | s 1 | SG 2 | 3 | GS 2 | SO 3 | 2 | SsG −3 | OSG +3 |
| BSS2 .. .. | G −1 | 3·6 | 2·2 | .. | s 1 | SG −2 | −3 | GS +2 | SO 2 | −2 | sG 2 | SG +2 |
| BSS3 .. .. | G −1 | 4·1 | 2·4 | 2·0 | s 1 | SG +1 | 3 | GS 2 | S +2 | −3 | SsG 2 | SG 2 |
| BSR1 .. .. | G 1 | 3·2 | 3·9 | 9·9 | Ss 2 | SsG +2 | 4 | SsG −4 | Sg 3 | −3 | SsG 4 | SsG +3 |
| BSR2 .. .. | G 1 | 1·5 | 2·2 | 28·0 | Ss 2 | SsG 3 | −3 | SsG +3 | SsG 2 | −3 | SsG 4 | SsG 4 |
| BSR3 .. .. | G 1 | 2·6 | 2·5 | 13·1 | Ss −2 | SsG 2 | 3 | SsG −4 | SsG 2 | +3 | SsG 4 | SsG 3 |
| CSR1 .. .. | G 1 | 2·5 | 3·5 | 8·6 | SG +2 | SsG 3 | −3 | SsG 3 | Ss −2 | −3 | SsG 3 | SsG 3 |
| CSR2 .. .. | G 1 | 2·5 | 4·1 | 11·6 | SG 2 | SsG −3 | 3 | SsG 3 | Ss +1 | −3 | SsG −4 | SsG −3 |
| CSR3 .. .. | G 1 | 3·9 | 3·7 | 10·2 | SG 2 | SsG +3 | 3 | SsG 4 | SsG 3 | .. | SsG 4 | SsG 4 |
| Average .. | 1 | | | | 1·4 | 2·2 | 2·8 | 2·9 | 2·3· | 2·6 | 2·9 | 2·8 |

S = Stale. G = Gluey. s = Scorched. O = Oxidized.

average $\frac{1}{3}$ grade unit better. From the data in Table 4, it may be seen that up to eight times the quantity of oxygen was absorbed and twice the amount of $CO_2$ evolved by the air-packed compared with the gas-packed samples after 40 weeks at 37°, but the average difference in grade did not exceed 0·4 of a unit. The types of off-flavour that developed in the gas-packed samples were identical with those developing in the corresponding air-packed powders, from which it may be concluded that the degree of stale, gluey, and scorched flavours developed is not directly proportional to the amount of oxygen absorbed.

TABLE 4.—OXYGEN ABSORBED AND $CO_2$ EVOLVED BY SKIM MILK POWDER STORED AT 37°C. FOR 40 WEEKS.

| Average Three Samples from Factory— | Percentage of Total Gas Space. | | | Grading. |
|---|---|---|---|---|
| | Original $O_2$. | $O_2$ Absorbed. | $CO_2$ Evolved. | |
| A spray .. .. .. | 3·6 | 2·3 | 0·8 | 2·0 |
| | 21 | 9 | 1·2 | 2·3 |
| B spray .. .. .. | 3·8 | 3·1 | 0·6 | 2·3 |
| | 21 | 7 | 1·1 | 2·7 |
| B roller .. .. .. | 1·5 | 0·9 | 0·4 | 3·0 |
| | 21 | 6 | 0·7 | 3·0 |
| C roller .. .. .. | 1·5 | 1 | 0·3 | 2·7 |
| | 21 | 8 | 1·0 | 3·0 |

Samples stored in friction-lid tins at 37° deteriorated at about the same rate as corresponding samples sealed in tins containing air. The moisture content increased by about 1 per cent. during storage for 40 weeks.

(iv) *Keeping Quality of Whole Milk Powders.*

(*a*) *Influence of temperature of storage.*—Representative data are summarized in Table 5. These samples were single gas-packed. More rapid deterioration occurred with increasing temperature, but the differences, particularly between samples stored at 15° and 30°, are not striking. Average figures for the six samples from Factories A and D are given at the foot of the table, from which it may be seen that after 26, 39, and 61 weeks there is no significant differences between samples stored at 15° and 30°, whereas at 30° and 37° differences of the order of 1 grade unit were noted after 26 and 39 weeks. Average figures for all samples also show comparatively small differences between samples stored at 15° and 30° but a more marked effect between samples stored at 30° and 37°.

(*b*) *Keeping quality of gas-packed whole milk powders stored at* 15°, 30°, *and* 37°*C.*—Samples for which data are given in Table 5 were gas-packed in the laboratory to 3·5 per cent. oxygen in the head-space for Series 1 and 3 spray-dried powders, and 1·3 per cent. for Series 1 and 3 roller-dried powders. Series 2 powders were not regassed but placed in store as received from the factories.

Spray-dried powders from Factories A and D kept considerably better than spray-dried powders from Factory B and roller-dried powders from Factories E and B. The samples from Factories E and

TABLE 5.—EFFECT OF STORAGE TEMPERATURE ON GAS-PACKED WHOLE MILK POWDERS.

| Sample. | Grade before Storage. | Moisture. | Cu. | Fe. | Head-space. | 26 Weeks. | | | | | | 39 Weeks. | | | | | | 61 Weeks. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 15.° | | 30.° | | 37.° | | 15.° | | 30.° | | 37.° | | 15.° | | 30.° | |
| | | % | μg/g. | μg/g. | | *a* | *b* | *a* | *b* | *a* | *b* | *a* | *b* | *a* | *b* | *a* | *b* | *a* | *b* | *a* | *b* |
| AFS1 | 0 | 3·0 | 0·8 | 1·4 | 3·5 | S1 | 1 | S+1 | +1 | S2 | 2 | S2 | 2 | SG+2 | +2 | O1<br>SG3 | 3 | O3 | 3 | S−1 | −1 |
| AFS2 | 0 | 2·8 | 1·3 | 2·4 | 3·5 | O1<br>S1 | 2 | S+1 | +1 | O1<br>S3 | 3 | O1<br>S3 | 3 | O1<br>S2 | 2 | O1<br>S2 | 3 | O1<br>SG2 | −3 | G−3 | −3 |
| AFS3 | 0 | 2·8 | 1·4 | 2·5 | 3·6 | .. | .. | SG1 | 1 | O−1<br>S2 | 2 | S+1 | +1 | O1<br>S2 | +2 | O1<br>S2 | +2 | S1 | 1 | G−3 | −3 |
| DFS1 | 0 | 2·7 | 0·36 | 2·0 | 3·5 | .. | O | S1 | 1 | O1<br>S2 | 2 | S1 | 1 | S+1 | +1 | O1<br>S2 | −3 | S−2 | −2 | S1 | 1 |
| DFS2 | 0 | 2·2 | 0·30 | 3·1 | 5·5 | .. | .. | O1<br>S1 | 1 | .. | .. | O1<br>S−2 | −2 | Ss1 | 1 | S−2 | −2 | O1<br>S2 | +2 | S−1 | −1 |
| DFS3 | 0 | 2·6 | 0·39 | 2·9 | 3·5 | O1 | 1 | S1 | 1 | S2 | 2 | S1 | 1 | S−2 | −2 | O1<br>S2 | −2 | Ss1 | 1 | O1<br>S2 | 2 |
| BFS1 | Ss 1 | 2·0 | 2·8 | 2·5 | 3·5 | S+2 | +2 | G−2 | −2 | .. | .. | .. | .. | .. | .. | SG4 | 4 | SsG | 4 | O+4 | +4 |
| BFS2 | Ss 1 | 2·6 | 2·7 | 4·0 | 8·5 | .. | .. | .. | .. | .. | .. | .. | .. | O1<br>S+3 | +3 | O+4<br>S3 | +4 | O4<br>S4 | +4 | O+4 | +4 |
| BFS3 | Ss 1 | 2·1 | 2·3 | 3·6 | 3·5 | S+2 | +2 | GS2 | 2 | .. | .. | .. | .. | O−1<br>S−3 | −3 | O2<br>S2 | 3 | S4 | 4 | O1<br>SG4 | 4 |
| EFR1 | s 1 | 1·9 | 0·46 | 14·0 | 1·3 | Ss+1 | +1 | Ss2 | 2 | Ss 3 | 3 | SsG−2 | −2 | Ss−4 | −4 | SsG3 | 3 | sG3 | 3 | O−4<br>SG−4 | −4 |
| EFR2 | s 1 | 1·9 | 0·48 | 13·0 | 2·5 | O1<br>S2 | 2 | O1<br>Ss2 | 2 | Ss +2 | +2 | O1<br>SsG3 | 3 | O4<br>Ss4 | +4 | O+4<br>S3 | +4 | sG3 | 3 | SG−3 | −3 |
| BFR2 | s 1 | 3·2 | 1·1 | 10·7 | 7·0 | S+1 | +1 | O1<br>Ss−3 | −3 | SsG+2 | +2 | .. | .. | .. | .. | O2<br>S+3 | +3 | O1<br>SG2 | 3 | S3 | 3 |
| BFR3 | 0 | 2·7 | 1·6 | 14·5 | 1·3 | S | 2 | Ss2 | 2 | S2 | 2 | O1<br>SG2 | 3 | O1<br>G2 | 2 | O−1<br>Ss+2 | −3 | O1<br>SG2 | 3 | O−1<br>SsG−4 | −4 |
| Average all samples .. .. | | | | | | | 1·5 | | 1·6 | | 2·25 | | 2·0 | | 2·4 | | 3·0 | | 2·8 | | 2·8 |
| Average first six samples .. | | | | | | | 1·0 | | 1·1 | | 2 | | 1·7 | | 1·7 | | 2·5 | | 2·0 | | 1·8 |

S = Stale. s = Scorched. G = Gluey. O = Oxidized. *a* = Off-flavours and their relative significance according to the grading plan. *b* = Overall grade score.

B showed slight off-flavours before they were placed in store and throughout storage remained about 1 to 1·5 grade points worse than the samples from Factories A and D. The better quality spray-dried samples showed no more than slight deterioration after six months at 30°, while some samples were of good quality after twelve months. After fifteen months at 15° and 30°, some of the samples from Factories A and D still showed only slight off-flavours, while most of the roller powders and spray powder from Factory B were unusable. Some at least of the differences between the keeping quality of the powders from Factories E and B compared with Factories A and D were probably due to the high copper or iron content of the powders from the former factories.

The data are too few to indicate the effect if any between samples from the various factories repacked to 3·5 per cent. oxygen in the headspace (Series 1 and 3) and the Series 2 powders which were not repacked. The few figures given in Table 5 tend to show that no great differences could be expected between samples gas-packed to 3·5 per cent. and 6–8 per cent. $O_2$.

(*c*) *Effect of gas-packing on the keeping quality of whole milk powders stored at* 37°C.—The data for Series 1 whole milk powders are given in Table 6. Data for Series 2 and 3 were similar and are therefore not given in detail.

Compared with storage in air at 37°, gas-packing to 3·5 per cent. oxygen in the headspace gas for spray-dried powders and to 1·3 per cent. for roller-dried powders very greatly reduced the rate at which oxidized flavours appeared and the intensity of these flavours at the end of 40 weeks' storage. Strong oxidized flavours were present in most samples packed in air after ten weeks' storage, whereas only slight oxidized flavours were detected in gas-packed spray-dried powders after 40 weeks. No oxidized flavours were detected in roller-dried powders gas-packed to 1·3 per cent. oxygen in the headspace gas, except in the samples from Factory B after 40 weeks' storage when a suspicion of oxidized flavour was noted. This explains why no extra keeping quality was conferred on the roller-dried powders by gassing to 0·3 per cent. oxygen. With the spray-dried powders, however, where gassing to 3·5 per cent. oxygen was not fully effective in preventing oxidized flavours from appearing, additional keeping ability was noted in samples gassed to 0·6 per cent. oxygen. At this oxygen level no oxidized flavours appeared even when all the oxygen had been absorbed.

(*d*) *The relative keeping qualities of skim milk and whole milk powders.*—This section has been included because of previous lack of evidence as to whether skim milk powder when reconstituted after storage with fresh flavoured butterfat gave a liquid of better flavour than whole milk powder stored under the same temperature conditions. The comparison has been carried out on skim and whole milk powders as normally packed commercially: i.e., the skim milk packed in friction-lid tins and the whole milk gas-packed to 3·5 per cent. oxygen in the headspace gas. Duplicate tins of the whole milk powders gas-packed to 0·6 per cent. oxygen were included. As roller-dried powders did not keep as well as the spray-dried powders, only the data for the latter have been used. Also, as the whole milk powder from Factory B was

TABLE 6.—EFFECT OF GAS PACKING ON WHOLE MILK POWDERS STORED AT 37°.

| Sample. | $O_2$ Original Head-space. | 4 Weeks. | | | 10 Weeks. | | | 14 Weeks. | | | 24 Weeks. | | | 40 Weeks. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $O_2$ Used. | Grading. | | $O_2$ Used. | Grading. | | $O_2$ Used. | Grading. | | $O_2$ Used. | Grading. | | $O_2$ Used. | Grading. | |
| | % | % | *a* | *b* | % | *a* | *b* | % | *a* | *b* | % | *a* | *b* | % | *a* | *b* |
| AFS1 .. .. | 21 | 1·0 | | O | 4·1 | S2 | 2 | 4·7 | O1<br>S+1 | 2 | 8·8 | O+4<br>S2 | +4 | 11·5 | O+4<br>S2 | +4 |
| | 3·5 | 0·21 | | O | 0·79 | S1 | 1 | 1·10 | SG−1 | −1 | 2·33 | O1<br>S3 | 3 | 2·50 | O1<br>SG3 | 3 |
| | 0·6 | 0·0 | | O | .. | .. | .. | 0·19 | SG1 | 1 | 0·52 | S2 | 2 | 0·60 | S+2 | +2 |
| DFS1 .. .. | 21 | 2·3 | S−1 | −1 | 4·0 | O3 | 3 | 6·4 | O4<br>S2 | 4 | 9·6 | O+4<br>S2 | +4 | 20·6 | O+4<br>S2 | +4 |
| | 3·5 | 0·08 | | O | 0·47 | S1 | 1 | 0·96 | S1 | 1 | 1·75 | O−1<br>S2 | 2 | 2·58 | O1<br>S2 | −3 |
| | 0·6 | 0·0 | | O | 0·28 | S−1 | −1 | 0·26 | Ss1 | 1 | 0·41 | S−1 | +1 | 0·60 | S+1 | +1 |
| BFS1 .. .. | 21 | 2·7 | S1 | 1 | 4·5 | O4 | 4 | 6·7 | O+4 | +4 | 7·0 | .. | .. | 15·1 | O+4<br>S2 | +4 |
| | 3·5 | 0·36 | S1 | 1 | 1·18 | O1<br>S2 | 2 | .. | .. | .. | 1·80 | .. | .. | 3·25 | SG4 | 4 |
| | 0·6 | 0·21 | SG2 | 2 | 0·34 | S2 | 2 | 0·60 | SG−2 | −2 | 0·35 | .. | .. | 0·60 | SG4 | 4 |
| EFR1 .. .. | 21 | 1·9 | O1<br>Ss2 | 3 | 4·8 | O+4 | +4 | 8·1 | O4<br>Ss3 | 4 | 20·3 | .. | .. | 20·0 | O−4<br>SsG3 | +4 |
| | 1·3 | 0·0 | Ss+2 | +2 | 0·60 | Ss2 | 2 | 0·80 | Ss3 | 3 | 0·31 | .. | .. | 1·20 | SG4 | 4 |
| | 0·3 | 0·0 | Ss3 | 3 | 0·12 | Ss−2 | −2 | 0·26 | Ss2 | 2 | 0·13 | .. | .. | 0·30 | SG4 | 4 |
| BFR1 .. .. | 21 | 1·8 | sG2 | 2 | 3·8 | O4<br>S2 | 4 | 5·4 | O+4 | +4 | 15·8 | .. | .. | 20·9 | O+4<br>S2 | +4 |
| | 1·3 | 0·38 | sG2 | 2 | 0·48 | Ss2 | 2 | 1·01 | S2 | 2 | 1·10 | .. | .. | 1·30 | O−1<br>Ss2 | −3 |
| | 0·3 | 0·22 | sG2 | 2 | 0·25 | Ss2 | 2 | 0·25 | S2 | 2 | 0·22 | .. | .. | 0·30 | O−1<br>Ss+2 | −3 |

S = Stale G = Gluey. s = Scorched. O = Oxidized. *a* = Off-flavours and their relative significance according to the grading plan. *b* = Overall grade score.

generally poor in quality compared with those from Factories A and D, only the powders from the latter factories have been compared with the spray-dried skim milk powders from Factories A and B. The comparison is of course seriously limited by the fact that the powders were made from different milk supplies. The data are summarized in Table 7.

Table 7.—The Relative Keeping Qualities of Skim Milk and Whole Milk Powders.

| — | $O_2$ in Head-space. | Original Grading. | After 26 Weeks at— | | | After 39 Weeks at— | | | After 61 Weeks at— | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 15°. | 30.° | 37.° | 15.° | 30.° | 37.° | 15.° | 30.° |
| *Whole milk—* | % | | | | | | | | | |
| AFS1 | 3·5 | 0 | 1 | +1 | 2 | 2 | +2 | 3 | 3 | −1 |
| | 0·6 | | .. | .. | +1 | .. | .. | +2 | .. | .. |
| AFS2 | 3·5 | 0 | 2 | +1 | 3 | 3 | 2 | 3 | −3 | −3 |
| | 0·6 | | .. | .. | 2 | .. | .. | 2 | .. | .. |
| AFS3 | 3·5 | 0 | .. | 1 | 2 | +1 | +2 | +2 | 1 | −3 |
| | 0·6 | | .. | .. | +1 | .. | .. | 2 | .. | .. |
| DFS1 | 3·5 | 0 | 0 | 1 | 2 | 1 | +1 | −3 | −2 | 1 |
| | 0·6 | | .. | .. | +1 | .. | .. | +1 | .. | .. |
| DFS2 | 3·5 | 0 | .. | 1 | +1 | −2 | 1 | −2 | +2 | −1 |
| | 0·6 | | .. | .. | +1 | .. | .. | −2 | .. | .. |
| DFS2 | 3·5 | 0 | 1 | 1 | +1 | 1 | −2 | −2 | 1 | 2 |
| | 0·6 | | .. | .. | 1 | .. | .. | +1 | .. | .. |
| *Skim milk—* | | | | | | | | | | |
| ASS2 .. | 21 | −1 | −1 | 1 | 3 | 2 | −2 | 2 | +1 | +1 |
| ASS3 .. | 21 | −1 | −1 | 1 | +1 | 3 | 2 | −3 | +1 | 2 |
| BSS1 .. | 21 | −1 | 1 | 2 | 3 | 2 | 3 | 3 | −3 | +3 |
| BSS2 .. | 21 | −1 | 1 | −2 | −3 | +2 | 2 | 3 | 2 | +2 |
| BSS3 .. | 21 | −1 | 1 | +1 | 3 | 2 | +2 | −3 | 2 | 2 |

The most noticeable feature of the table is that the skim milk powders showed slight off-flavour compared with the whole milk powders at the time of placing in store, and for this reason whole milk powder would be preferred for at least the first few months of storage. After six months, considerable variation in quality of the samples from any one factory makes comparison difficult, but at all temperatures tried the whole milk powders gassed to 3·5 per cent. oxygen appeared to be slightly better than the skim milk powders. The whole milk powders gassed to 0·6 per cent. oxygen and stored at 37° were definitely better than the equivalent skim powders. After ten months' storage the same conclusions may be drawn. After fifteen months at 15° and 30° the figures are less conclusive. The worst sample was a skim milk powder and the best a whole milk, but the skim milk powders from Factory A compare favourably with the whole milk powders. Variations in the keeping qualities of samples from the one factory were greater than the differences between the two types of powder.

There was a tendency for the quality of the skim milk powders to improve between ten and fifteen months' storage. The differences noted may have been due to a change in the grading standard, but is thought that an actual improvement in flavour is a more likely explanation.

(*e*) *Useful life of spray-dried skim and whole milk powders.*—As there was little difference in keeping quality at 15° and 30°, the useful life under temperate and sub-tropical conditions of good quality skim and whole spray-dried milk powders may be assessed from Table 7. Powders grading 0 and 1 are considered to be good, 2 barely usable as liquid milk, 3 possibly usable for some cooking purposes, and 4 unusable. Up to six months air-packed skim milk powders and gas-packed (3·5 per cent. oxygen) whole milk powders were on the whole good (graded 0 and 1), but after ten months' storage the skim milk powders were barely usable as liquid milk, while some of the whole milk powders were still of good quality and others barely usable as liquid milk. After fifteen months' storage, about 50 per cent of the whole milk powders were still good while the remainder were usable only for cooking. Oxidized flavours were absent or slight, gluey and stale flavours being mainly responsible for the loss in grade. Skim milk powders from Factory A were just usable as liquid milk after fifteen months, while those from Factory B were slightly inferior, one of the three being usable only for cooking.

Thus the better brands of spray-dried skim and whole milk powders are reliable up to six months' storage. Although about half the samples from some of the factories were in good condition after fifteen months at 15° and 30°, others from the same factories had deteriorated considerably, so that the quality of these powders could not be relied upon after prolonged storage.

(v) *Bacteriological Examinations.*

Bacteriological examinations were undertaken in order to determine whether the bacterial content of the powders was related to their initial flavour and keeping quality. It was also thought desirable to obtain data which would enable some comparison to be made between Australian milk powders and those of other countries in respect of bacteriological quality.

(*a*) *Methods of examination.*—Methods of examination were substantially in accordance with those described by Crossley and Johnson (1942). A mechanical shaker was found more convenient and possibly slightly more accurate than the hand shaking suggested by these authors for dissolving the powders in the dilution blanks. Also, some difficulty was encountered in the reconstitution of roller-dried and stale spray-dried milks in tap water owing to the occurrence of numerous undissolved particles which in the agar were undistinguishable from colonies. The use of blanks containing various concentrations of lithium and sodium hydroxide to overcome this difficulty has been suggested, but it was found that at concentrations of these compounds sufficiently high to effect complete solution of the powders, the plate counts were appreciably reduced. Successful reconstitution with this type of powder was finally achieved by the use of sodium citrate. With 3·5 per cent. sodium citrate in tap water it was possible to obtain a perfectly clear plate even in the 1/100 dilution. Tested on fresh powders the sodium citrate blanks showed no reduction compared with tap water blanks either in total or thermophile counts. Preliminary warming of blanks to 40°C. was necessary to dissolve roller-dried powders.

All the whole milk powders taken for bacteriological examination were gas-packed, while the skim powders were from the series packed in air. The medium for total and thermophile counts was the standard tryptone-glucose-milk agar of the American Public Health Assoc. Total counts were incubated 72 hours at 37°C., thermophile counts 24 hours at 55°C. All results are presented on the basis of 1 g. of powder.

(*b*) *Results and discussion.*—Total and thermophile counts on the spray-dried powders are given in Tables 8 and 9. Results for the roller-dried powders are not given in detail as the counts were comparatively low and irregular. Initial total counts on these powders ranged from 700 to 33,000 per gram. The thermophile counts were uniformly below 1,000 per gram except in the E series of roller-dried whole milks, in which the two powders had counts of 101,000 and 125,000 per gram respectively.

TABLE 8.—TOTAL AND THERMOPHILE COUNTS ON SPRAY-DRIED WHOLE MILK POWDERS.

| Sample. | Type of Count. | Count per 1 g. of Powder. | | | |
|---|---|---|---|---|---|
| | | Initial. | 26 Weeks at 15°C. | 26 Weeks at 30°C. | 26 Weeks at 37°C. |
| AFS1 .. | Total 37°C. .. .. | 128,000 | 117,000 | 34,000 | not done |
| | Thermophile 55°C. .. | 1,800 | 300 | 2,300 | not done |
| AFS2 .. | Total 37°C. .. .. | 644,000 | 544,000 | 380,000 | 160,000 |
| | Thermophile 55°C. .. | 1,500 | 1,000 | 1,500 | 1,200 |
| AFS3 .. | Total 37°C. .. .. | 690,000 | 420,000 | 174,000 | 102,000 |
| | Thermophile 55°C. .. | 400 | 800 | 2,800 | 2,000 |
| BFS1 .. | Total 37°C. .. .. | 71,000 | 34,000 | 13,000 | 6,200 |
| | Thermophile 55°C. .. | 27,200 | 12,700 | 9,100 | 8,600 |
| BFS2 .. | Total 37°C. .. .. | 270,000 | 98,000 | 16,300 | 3,500 |
| | Thermophile 55°C. .. | 3,800 | 4,000 | 6,500 | 7,400 |
| BFS3 .. | Total 37°C. .. .. | 31,000 | 13,000 | 6,700 | 500 |
| | Thermophile 55°C. .. | 300 | 100 | 1,000 | 700 |
| DFS1 .. | Total 37°C. .. .. | 1,400 | 3,800 | <100 | not done |
| | Thermophile 55°C. .. | 800 | <100 | <100 | not done |
| DFS2 .. | Total 37°C. .. .. | 83,000 | 54,000 | 14,000 | 4,800 |
| | Thermophile 55°C. .. | 600 | 1,000 | 1,700 | 100 |
| DFS3 .. | Total 37°C. .. .. | 4,800 | 2,500 | 1,400 | 200 |
| | Thermophile 55°C. .. | <100 | <100 | 600 | 400 |

The total counts on all samples showed a consistent decrease after storage, the rate of decrease being regularly more pronounced at the higher temperatures. With thermophilic organisms the same tendency was not shown, except in the few instances in which large numbers were encountered. Although the number of samples examined was not large,

TABLE 9.—TOTAL AND THERMOPHILE COUNTS ON SPRAY-DRIED SKIM MILK POWDERS.

| Sample. | Type of Count. | Count per 1 g. of Powder. | | | |
|---|---|---|---|---|---|
| | | Initial. | 26 Weeks at 15°C. | 26 Weeks at 30°C. | 26 Weeks at 37°C. |
| ASS1 .. | Total 37°C. .. .. | 1,700,000 | 1,190,000 | 704,000 | not done |
| | Thermophile 55°C. .. | 3,400 | 7,100 | 6,300 | not done |
| ASS2 .. | Total 37°C. .. .. | 968,000 | 756,000 | 360,000 | 71,000 |
| | Thermophile 55°C. .. | 5,100 | 2,300 | 3,900 | 4,700 |
| ASS3 .. | Total 37°C. .. .. | 760,000 | 580,000 | 241,000 | 164,000 |
| | Thermophile 55°C. .. | 5,900 | 10,900 | 6,100 | 24,400 |
| BSS1 .. | Total 37°C. .. .. | 112,000 | 13,800 | 3,400 | not done |
| | Thermophile 55°C. .. | 223,000 | 180,000 | 246,000 | 37,000 |
| BSS2 .. | Total 37°C. .. .. | 46,000 | 13,900 | 4,300 | 1,000 |
| | Thermophile 55°C. .. | 18,000 | 7,000 | 11,400 | not done |
| BSS3 .. | Total 37°C. .. .. | 39,000 | 8,000 | 2,600 | 1,100 |
| | Thermophile 55°C. .. | 2,900 | 1,100 | 1,900 | 1,500 |

the total counts of the spray-dried powders tended to be associated with the factory of origin. Samples from Factory A for instance showed a consistently high and from Factory D a consistently low level of counts.

(*c*) *Types of organisms present.*—A clear cut differentiation was observed between the flora of spray-dried and roller-dried powders. In the former the organisms observed consisted almost entirely of streptococci with only a very few micrococcus and sarcina forms. In the roller-dried powders, the organisms present were predominantly micrococci, sarcinae, and sporing bacteria, with very few streptococci. Fifty-five colonies of streptococci were selected at random from plates of spray-dried powders. They were classified on the basis suggested by Sherman (1937); 36 colonies, or 65 per cent., belonged to the "enterococcus" group of streptococci, and 19, or 35 per cent., to the "viridans" group. None of the "lactis" group was isolated.

A large proportion of the fresh powders examined (particularly the spray-dried skim powders) gave positive coliform tests based on 2 g. samples. Tests on 0·1 g. samples were almost uniformly negative. Powders after storage showed almost entirely negative coliform results even with 2 g. samples. In only two samples (roller-dried powders) were yeast colonies observed even in the 1/10 dilution. Mould colonies ranged from 10 to 60 per g. in the fresh powders. They tended to increase generally to between 50 and 250 colonies per g. in the case of powders stored at 15°C. There was no tendency to increase at 30° and 37°C.

(*d*) *Relation between bacterial counts and quality of powders.*—No consistent differences were observed either in respect of initial flavour or keeping quality which could be correlated with the bacteriological condition of the powders. The possibility that biological factors

are concerned in the determination of milk powder quality cannot be excluded, but it is obvious that their significance has been obscured in the present instance by various chemical and physical effects.

Owing to the relatively small number of samples examined, adequate comparison with the results of other investigators is not possible. The results, however, in regard to the range of total and thermophile counts, the types of organisms encountered, and the effect of storage on plate counts, generally appear to be consistent with those obtained by Nichols (1939) and Crossley and Johnson (1942) from the examination of various English milk powders.

(vi) *Comparison with English Results.*

The results obtained by Lea *et al.* (1943) on stored samples of roller-dried and spray-dried whole milk powders differ somewhat from the results of the present experiment. Stale and gluey flavours in their experience did not seem to play such a large part in the deterioration of gas-packed samples, and accelerated storage tests at 47° and 37° enabled them to predict reasonably accurately the expected storage life at 15°. They found that tallowiness in milk powder had a temperature coefficient of about 2 per 10°. As mentioned in an earlier section, loss of grade due to tallowiness was usually unimportant compared with stale and gluey flavours in samples gas-packed to 3·5 per cent. oxygen in the headspace gas, and moreover, stale and gluey flavours seemed to develop almost as rapidly at 15° as at 30° and only slightly more rapidly at 37°. Lea *et al.* mention noticing that slight stale flavours were sometimes detected in samples stored at —20° and 15°, while the corresponding samples stored at higher temperature were unchanged or slightly stale, and they draw the inference that high-temperature accelerated tests may not be valid for this particular defect. This may explain why the effect of temperature was slight in the present work. It does not explain why staleness was so marked in the Australian milk powders, while almost absent in the English powders. Moisture content is known to influence the development of staleness (Supplee and Bellis, 1925), but the powders studied by Lea *et al.* contained 1·3 to 4 per cent. moisture, and this range covers the moisture content of the Australian powders.

## 5. Conclusions.

(1) Skim milk powders when reconstituted after storage with fresh flavoured butterfat were no better in quality than gas-packed whole milk powders stored under similar conditions of temperature. During the first few months of storage the whole milk powders were invariably preferred because the skim milk powders showed slight stale and gluey flavours on arrival from the factories.

(2) Gas-packing had only a slight effect in increasing the keeping quality of skim milk powders, although considerable amounts of oxygen were absorbed by powders packed in air.

(3) Spray-dried powders, both skim and whole milk, kept better than the corresponding roller-dried powders.

(4) Skim milk powders after six months at 15, 30, and 37°C. showed considerable variation in quality, but after nine months there was little to choose between samples stored at the three temperatures.

(5) Gas-packed whole milk powders were also surprisingly little affected by storage temperature. At 15 and 30°C. there were no significant differences, but at 37°C. the quality fell off more rapidly than at 30°.

(6) Gas packing to 3·5 per cent. oxygen in the headspace gas of tins of spray-dried whole milk powders greatly increased the life of these samples compared with those stored in air. Oxidized flavours were not entirely eliminated at this oxygen tension, but roller-dried powder gas-packed to 1·5 per cent. oxygen, and spray-dried powders double gas-packed to 0·6 per cent. were free from this defect.

(7) Stale and gluey flavours were mainly responsible for loss of grade in gas-packed whole milk powders and also skim powders whether gas-packed or stored in air. These flavours limited the life of powders to about six months at 15° and 30°C., for, although many powders kept well up to fifteen months at 15° and 30°, other samples from the same factories had deteriorated extensively in this time.

(8) Variable keeping quality in different samples from individual factories was observed. This was not obviously related to differences in bacteriological counts or to moisture, copper, or iron contents.

(9) From 20 to 40 per cent. of the tins that had been gassed were found to be leaking. Faulty sealing technique at the milk drying factory was responsible.

## 6. Acknowledgments.

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# Studies on Compressed Whole Milk Powder.

*By C. C. Thiel, B.Sc (Agric.), Ph.D.**

*Summary.*

Compression of spray-dried whole milk powder to a density of 1·15 to 1·20 reduced the interstitial oxygen as effectively as the usual gas-packing in tins, but neither cellophane nor waxed paper wrapping prevented uptake of atmospheric oxygen or moisture. Blocks containing 20 per cent. of cane sugar were much more friable and kept better than those made entirely of milk powder. Vanillin also improved the keeping quality of the blocks.

## 1. Introduction.

Early in 1941 an investigation was undertaken of the possibility of shipping wholemilk powder in compressed form so that both tinplate and shipping space might be conserved. After preliminary work to determine suitable operating conditions for the production of blocks containing minimal quantities of residual air, a number of 20-oz. blocks were subjected to keeping quality tests (Section 3 of this paper). Later, keeping quality tests on tablets to which either cane sugar or vanillin had been added were carried out to determine whether they were of use to the Armed Forces operating in tropical areas (Section 4).

If compression into block form is to be an adequate substitute for the usual process of gas-packing powder contained in tins, the blocks must be sufficiently strong to withstand normal handling, they must be protected from light, moisture, and atmospheric oxygen, and in addition the original amount of oxygen in the interstices of the block must be low. Protection from light was readily obtained by using an opaque final wrapper, but exclusion of moisture vapour and atmospheric oxygen presented considerable practical difficulties. Waxed paper and heat-sealing cellophane were experimented with, but other wrapping materials such as vinyl resin and rubber-base sheets were not available at the time these experiments were made.

## 2. General Considerations of the Compression of Dried Whole Milk Powder.

The oxygen content of compressed dried milk blocks is most readily calculated from the density of the block determined by weighing and measuring, and the true density of the milk solids. Stamberg and Bailey (1940) determined the true density of dried skim milk using a pycnometer method, and found it to be 1·470 for roller-dried and 1·459 for spray-dried powder. Assuming a density of 0·930 for butter-fat, then the true density of whole milk powder containing 26 per cent. fat and 3 per cent. water would be 1·31. This agrees with the values ranging from 1·31 to 1·32 obtained by Lea, Moran, and Smith (1943), using a gas densitometer. The density used in calculations in this paper was 1·31.

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(i) *Factors Determining the Final Density of Compressed Whole Milk Powder.*

The effects of pressure on final densities of blocks of roller-dried and spray-dried whole milk powder are shown graphically in Fig. 1. It will be noticed that much lower pressures are needed to compress roller-dried powder to a particular density than spray-dried powder, but that fat is pressed out from the blocks of roller-dried powder at a density of about 1·10, whereas a density of 1·18 was reached with the blocks of spray-dried powder before free fat appeared. Different samples of powder attained different final densities at a particular pressure, and with different samples loss of fat occurred at different pressures; the data given in Fig. 1 were obtained using samples that had approximately average compression characteristics.

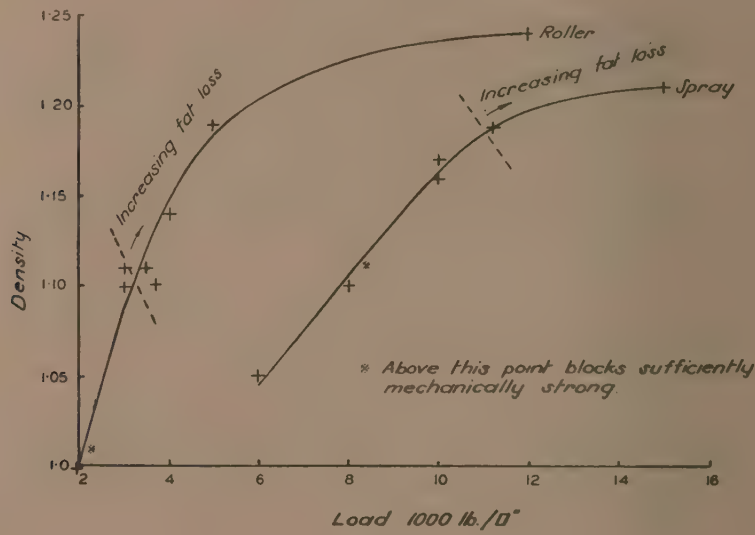


Fig. 1.

Compression of milk powder compares favourably with gas-packing from the point of view of removal of oxygen. A sealed tin of whole milk powder of bulk density 0·60, gas-packed to 3 per cent. final oxygen concentration in the headspace gas, would contain 0·027 ml. of oxygen per gram of milk powder. A block of compressed powder of density 1·10 would contain 0·030 ml. of oxygen per gram, and at a density of 1·18, 0·017 ml. of oxygen per gram.

It has been stated by Kellner (1937) that in compressing milk powder the pressure should be increased slowly, otherwise the pores of the block close and no more air can escape. At slow rates of loading, compared with very slow loading, this effect was not detected. Holding for a long time under pressure only removes a very small additional amount of air, which confirms the results reported by Lea *et al* (1943).

At 3 to 4 tons per square inch pressure, the blocks of spray-dried milk powder were soft and easily disintegrated by hand. At 5 to 6 tons per square inch the blocks were so hard as to require mechanical crushing and grinding. Mixing ordinary table sugar with the powder in the ratio of 1 part of sugar to 4 parts of powder by weight before compressing made the blocks much more friable. At pressures up to 5,000 lb./sq. inch such blocks could be disintegrated readily by hand.

(ii) *Production of Compressed Whole Milk Powder.*

Blocks of compressed milk have been successfully prepared using a variety of presses and dies. The 20-oz. cylindrical blocks used for storage tests (Section 3) were made in a steel die, using a materials testing press. The die was 9 inches high and 3·2 inches in diameter. It was made from solid drawn steel tubing of $\frac{1}{2}$ inch wall thickness, and the lower 3 inches of the bore was tapered so that the diameter of the lower opening was 3·375 inches.

At various times a variety of sizes of compressed milk blocks has been made by sawing large blocks (9 in. by 9 in. by 11 in. high approximately). These blocks were compressed in a 1,500-ton press normally used for the manufacture of salt stock-licks. For making 1-lb. and 2-lb. blocks the large blocks were sawn on a bandsaw; for 1-oz. tablets (see Section 4) an 8-gang saw was used as well as the bandsaw. Dust and off-cuts were repressed. For the smaller sizes of block, reciprocating presses would probably be satisfactory, and the small blocks would be more cheaply produced in this way than by sawing large blocks.

The round tablets of compressed milk used for storage experiments described in Section 4 were made with a rotary tableting machine normally used for making peppermint sweets. As milk powder in its usual form would not feed into the dies properly, the powder was first compressed into blocks and then ground between differential rolls. Using this expedient sufficient tablets for experimental purposes were readily produced.

### 3. Keeping Quality of Blocks of Milk Powder.

(i) *Introduction.*

Bulk samples of spray-dried whole milk powder from two factories were compressed into 1·25-lb. cylindrical blocks to a density of 1·19. The powder from Factory A required a pressure of 10,000 lb./sq. inch to compress it to this density, and the powder from Factory B 12,000 lb./sq. inch. Three types of wrapping were used—heat-sealing cellophane, absorbent paper followed by dipping in a mixture of 85 per cent. match wax and 15 per cent. ceresine wax at 95°C., and a combination of these wrappings with the cellophane on the inside.

Blocks were weighed and then stored at 37°C. and the humidity of the incubator, 30°C. and 70 to 80 per cent. relative humidity, and room temperature and humidity. Gas-packed 12-oz. tins of powder were used as controls at the various temperatures.

(ii) *Methods of Analysis.*

Each block after unwrapping was examined for surface cracking, and was then weighed to the nearest gram so that the percentage increase in weight could be calculated. Three samples were then taken from the

block—the surface layer to a depth of about 5 mm., a sample from the interior at least 1 cm. from the surface, and a radial sample that was representative of the whole block. These samples were then passed twice through a large type household mincer and then through a 45-mesh screen to remove unground particles. Under the microscope the ground and screened material appeared to be about as finely divided as the uncompressed powder. About 25 g. of powder was collected from each sample.

(iii) *Odour and Flavour.*

A 10-g. sample of powder was dissolved in 80 ml. water, and the reconstituted milk was held for 3 to 4 hours before tasting.

(iv) *Solubility.*

The method of Howat *et al.* (1939) was slightly modified, so that a larger sample could be taken and the laborious wetting of the sample by stirring with a rod avoided. In the modified method, 49·5 ml. of water was pipetted into a 6 in. by 1 in. Pyrex boiling tube, and 5·5 g. of milk powder added without stirring. When six tubes had thus been filled they were stoppered, and the powder in each then shaken down into the water by tapping the tubes vigorously on the hand until no more lumps could be seen. To break up any small lumps that may have formed and to thoroughly wet the powder, the tubes were then shaken for one minute in a mechanical shaker at the rate of 275 double excursions per minute with an amplitude of 1¼ inch. The tubes were then held for five minutes at 20° C. or 50° C. and then shaken for a further one minute. Some of the liquid was then centrifuged at 2,000 r.p.m. for two minutes (distance from centre of gyration to the middle of the centrifuge tube 10 cm.) and total solids, including fat, determined on the supernatant material as in the method of Howat *et al.* The solubility index was corrected for the moisture content of the milk powder.

From the results listed in Table 1 it may be seen that duplicate determinations of solubility using the modified method agree reasonably well. The figures refer to the gas-packed powders used as controls in the storage experiments at room temperature and 30°C., and were accumulated over a period of twelve months.

Table 1.—Duplicate Determinations of Solubility at 20°C. and 50°C. of Various Spray-Dried Whole Milk Powders.

| Sample. | Solubility at 20°C. | Solubility at 50°C. |
|---|---|---|
| | % | % |
| 1 | 97·9 | 99·6 |
| | 97·9 | 99·9 |
| 2 | 99·1 | 98·8 |
| | 99·2 | 98·6 |
| 3 | 99·1 | 99·3 |
| | 99·2 | 99·1 |
| 4 | 98·5 | 98·4 |
| | 98·3 | 98·6 |

(v) *Moisture.*

This was determined on a 1-g. sample by drying to constant weight in a vacuum oven at 100° C. and a residual pressure equivalent to about 2 inches of mercury. Air dried by bubbling through concentrated sulphuric acid was admitted to the interior of the oven at the rate of two bubbles per second. The aluminium dishes were 2½ inches in diameter and ½ inch deep, with lids similar to those on petri dishes. Constant weight was obtained in about six hours.

(vi) *Results.*

The blocks stored at 37°C. were examined after storage for 5, 9, 12, and 18 weeks; at 30°C. after 12, 26, and 38 weeks; and at room temperature after 12, 26, 38, and 50 weeks. The data obtained are summarized in Tables 2, 3, and 4.

After nine weeks at 37°C. the blocks showed little deterioration, but after storage for twelve weeks most blocks were inferior to the controls. At 30° C. (75 to 80 per cent. relative humidity) and also at room temperature, flavour changes had occurred by the first examination (after twelve weeks). At all temperatures, samples from the outsides of the blocks deteriorated much more extensively than the samples taken from the interiors of the blocks. In fact, at both room temperature and 30°C. the inside samples showed hardly any additional deterioration after the first three months, whereas the outside samples became progressively more stale, and in many cases oxidized. Also, the outside samples became progressively insoluble, while the interiors of the blocks showed only slightly decreased solubility.

Cellophane-wrapped blocks tended to show surface cracking and discolouration, while the waxed blocks and double-wrapped blocks remained free from these defects. Cracking and browning were associated with rates of increase in moisture content, which was about twice as high for cellophane as for waxed or double-wrapped blocks. At 30°C. the relative humidity was maintained at 70 to 80 per cent. for the first six months, after which it was reduced to 20 per cent. At the low humidity the blocks began to lose water (Table 3).

(vii) *Conclusions.*

From this experiment with two samples of spray-dried whole milk powder compressed into very hard blocks, it may be tentatively concluded that compression and packaging in heat-sealing cellophane and/or waxed paper is not an adequate substitute for gas-packing in tins. The surfaces of the blocks suffered loss in solubility and became stale, and in many cases oxidized. Thus the wrappings used were not effective barriers to either moisture or oxygen over long periods. However, uptake of moisture was probably a much more serious cause of deterioration than oxygen, so that if the blocks were packed in sealed tins they might be expected to have a life comparable with that of gas-packed powder.

TABLE 2.—KEEPING QUALITY OF COMPRESSED WHOLE MILK POWDER HELD IN 37°C. INCUBATOR.

| Wrapping. | | 1st Examination, Five Weeks. | | | 2nd Examination, Nine Weeks. | | | 3rd Examination, Twelve Weeks. | | | 4th Examination, Eighteen Weeks. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Gain in Weight. | Surface Cracks. | Grading. | Gain in Weight. | Surface Cracks. | Grading. | Gain in Weight. | Surface Cracks. | Grading. | Gain in Weight. | Surface Cracks. | Grading. |
| Brand A— | | % | | | % | | | % | | | % | | |
| Cello... | Ins... | −0·4 | Few small | Fresh | 0·2 | Mod. number 1–2 mm. deep | Fresh .. | 0·2 | Many small cracks | Fresh .. | 0·2 | Mod. number cracks | Stale |
| | Out. | .. | .. | Fresh | .. | .. | Fresh .. | .. | .. | Sl. tallowy, sl. cardb. | .. | .. | Stale |
| | Comp. | .. | .. | Fresh | .. | .. | Fresh .. | .. | .. | Sl. stale .. | .. | .. | Stale |
| Wax .. | Ins... | −0·2 | Very sl... | Fresh | 1·2 | Very sl. .. | Fresh .. | 1·2 | Nil .. | V. sl. stale | 1·2 | Nil .. | V. stale |
| | Out. | .. | .. | Fresh | .. | .. | Sl. stale | .. | .. | Cardb., tallowy | .. | .. | V. stale |
| | Comp. | .. | .. | Fresh | .. | .. | Fresh .. | .. | .. | Sl. cardb... | .. | .. | V. stale |
| Wax + Cello. | Ins... | −0·6 | Nil .. | Fresh | 0·0 | Nil .. | Fresh .. | 0·4 | Nil .. | Fresh .. | 0·2 | Nil .. | Stale |
| | Out. | .. | .. | Fresh | .. | .. | Fresh .. | .. | .. | V. v. sl. stale | .. | .. | Stale |
| | Comp. | .. | .. | Fresh | .. | .. | Fresh .. | .. | .. | V. sl. stale | .. | .. | Stale |
| Brand B— | | | | | | | | | | | | | |
| Cello... | Ins... | −0·4 | Few small | Fresh | 0·4 | Mod. number of cracks | Fresh .. | 0·2 | Mod. number sm. cracks | Sl. stale .. | 0·6 | Few small cracks | Sl. stale |
| | Out. | .. | .. | Fresh | .. | .. | Sl. stale | .. | .. | Stale, cardb. | .. | .. | Stale |
| | Comp. | .. | .. | Fresh | .. | .. | Fresh .. | .. | .. | Sl. stale .. | .. | .. | Stale |

TABLE 2.—KEEPING QUALITY OF COMPRESSED WHOLE MILK POWDER HELD IN 37°C. INCUBATOR—*continued.*

| Wrapping. | | 1st Examination, Five Weeks. | | | 2nd Examination, Nine Weeks. | | | 3rd Examination, Twelve Weeks. | | | 4th Examination, Eighteen Weeks. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Gain in Weight. | Surface Cracks. | Grading. | Gain in Weight. | Surface Cracks. | Grading. | Gain in Weight. | Surface Cracks. | Grading. | Gain in Weight. | Surface Cracks. | Grading. |
| Brand B— | | % | | | % | | | % | | | % | | |
| Wax .. | Ins... | −0·2 | Nil .. | Fresh | 0·8 | Nil .. | V. sl. stale | 1·0 | Nil .. | Sl. stale .. | 0·6 | Nil .. | Sl. stale |
| | Out. | .. | .. | Fresh | .. | .. | Sl. stale | .. | .. | Tallowy, cardb. | .. | .. | Stale |
| | Comp. | .. | .. | Fresh | .. | .. | Fresh .. | .. | .. | Stale, cardb. | .. | .. | Stale, sl. cardb. |
| Wax + Cello. | Ins... | −0·2 | Nil .. | Fresh | 0·2 | Nil .. | Fresh .. | 0·2 | Nil .. | Sl. stale .. | 0·2 | Nil .. | Sl. stale |
| | Out. | .. | .. | Fresh | .. | .. | Fresh .. | .. | .. | Unpleasant, stale cardb. | .. | .. | V. stale |
| | Comp. | .. | .. | Fresh | .. | .. | Fresh .. | .. | .. | Stale cardb. | .. | .. | Stale |
| Controls— | | | | | | | | | | | | | |
| A .. | .. | .. | .. | Fresh | .. | .. | Fresh .. | .. | .. | V. sl. stale | .. | .. | Sl. stale |
| B .. | .. | .. | .. | Fresh | .. | .. | Fresh .. | .. | .. | Sl. stale .. | .. | .. | V. sl. stale |

Ins. = Sample from interior of block. Out. = Sample of outer layers to depth of 5 mm. Comp. = Composite sample of interior and outside layers of the block.
Cello. = wrapped in heat-sealing cellophane. Wax = wrapped in paper and dipped in wax. Cello. + Wax = Combination of cello. and wax with the cellophane on the inside.

TABLE 3.—KEEPING QUALITY OF COMPRESSED WHOLE MILK POWDER HELD AT 30° C. AND 75 TO 80 PER CENT. R.H.

| Wrapping and Brand. | Increase in Weight of Blocks. | Surface Cracking. | Grading. | | | Percentage Solubility. | | | | | | Moisture. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1. | 2. | 3. | 1. | | 2. | | 3. | | 1. | 2. | 3. |
| | | | | | | 20.° | 50.° | 20.° | 50°. | 20.° | 50.° | | | |
| Brand A— | % | | | | | | | | | | | | | |
| Cello .. | (1) 1·3 | (1) Few large cracks | I. Sl. stale .. | Sl. stale .. | Sl. stale .. | 96 | 99 | 97 | 101 | 95 | 95 | 4·2 | 4·3 | 3·7 |
| | (2) 2·0 | (2) Sev. deep .. | O. V. strong cardb. | Stale .. | V. v. stale .. | 76 | 91 | 63 | 76 | 85 | .. | 4·5 | 5·8 | 4·6 |
| | (3) 0·6 | (3) Large number, extremely deep | C. Sl. cardb. .. | Stale .. | V. stale .. | 92 | 97 | 83 | 95 | 85 | 86 | 4·8 | 4·9 | 4·1 |
| Wax .. | (1) 0·8 | (1) Nil .. .. | I. Sl. stale .. | Sl. stale .. | Sl. stale .. | 96 | 99 | 97 | 100 | 94 | 96 | 3·1 | 3·8 | 3·9 |
| | (2) 1·2 | (2) A few fairly deep | O. Unpleasant metallic | Stale, tallowy | V. v. stale .. | 91 | 97 | 95 | 98 | 90 | 90 | 4·8 | 5·4 | 4·9 |
| | (3) 0·8 | (3) Few small .. | C. Sl. stale .. | Sl. stale .. | V. stale .. | 96 | 99 | 95 | 98 | 93 | 94 | 3·3 | 4·3 | 4·2 |
| Cello + Wax | (1) 0·4 | (1) Nil .. .. | I. Sl. stale .. | Sl. stale, watery | Stale .. | 94 | 100 | 98 | 101 | 94 | 98 | 3·4 | 3·9 | 4·0 |
| | (2) 0·4 | (2) Nil .. .. | O. Sl. cardb. .. | Stale .. | V. stale .. | 96 | 99 | 97 | 99 | 77 | 89 | 3·8 | .. | 4·3 |
| | (3) 0·6 | (3) Three long v. deep cracks | C. Sl. stale .. | Stale .. | Stale .. | 100 | 99 | 96 | 99 | 94 | 98 | 4·2 | 4·1 | 4·1 |
| Brand B— | | | | | | | | | | | | | | |
| Cello. | (1) 1·4 | (1) Sev. v. deep cracks | I. Stale .. | Sl. stale .. | Sl. stale .. | 95 | 98 | 95 | 98 | .. | 99 | 3·3 | 3·5 | 5·6 |
| | (2) 2·2 | (2) Many v. deep.. | O. Stale .. | V. stale cardb. | V. v. stale cardb. | 71 | 87 | 67 | 70 | 72 | 68 | 5·0 | 7·2 | 4·7 |
| | (3) 1·4 | (3) Many v. deep .. | C. Metallic .. | Stale cardb. | Stale oxidized | 97 | 92 | 91 | 92 | 89 | 91 | .. | 4·6 | 3·9 |

TABLE 3.—KEEPING QUALITY OF COMPRESSED WHOLE MILK POWDER HELD AT 30°C. AND 75 TO 80 PER CENT. R.H.—*continued.*

| Wrapping and Brand. | Increase in Weight of Blocks. | Surface Cracking. | Grading. | | | Percentage Solubility. | | | | | | Moisture. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1. | 2. | 3. | 1. | | 2. | | 3. | | 1. | 2. | 3. |
| | | | | | | 20.° | 50.° | 20.° | 50°. | 20.° | 50.° | | | |
| Brand B— | % | | | | | | | | | | | | | |
| Wax .. | (1) 0·8 | (1) Nil .. .. | I. Sl. stale .. | Sl. stale .. | Sl. stale .. | 98 | 99 | 99 | 100 | 96 | 97 | 3·5 | 3·5 | 3·7 |
| | (2) 1·2 | (2) Sev. deep .. | O. Stale .. | V. stale cardb. | V. v. stale cardb. | 97 | 100 | 90 | 95 | 88 | 93 | 5·7 | 5·7 | 4·4 |
| | (3) 0·6 | (3) Sev. v. deep .. | C. Stale .. | Stale cardb. | V. stale cardb. | 100 | 100 | 95 | 98 | 92 | 97 | 3·4 | 4·1 | 3·9 |
| Cello + Wax | (1) 1·0 | (1) Sev. deep cracks | I. Sl. stale .. | Sl. stale .. | V. stale .. | 98 | 99 | 97 | 99 | 97 | 98 | 3·6 | 4·0 | 3·6 |
| | (2) 0·6 | (2) Slight .. | O. Sl. stale .. | Stale cardb. | V. stale .. | 89 | 96 | 95 | 98 | 78 | 77 | 4·4 | 5·5 | 4·2 |
| | (3) 0·6 | (3) Many v. deep .. | C. Sl. stale .. | Stale .. | V. stale .. | 94 | 99 | 97 | 99 | 93 | 94 | 3·3 | 4·4 | 3·7 |
| Controls— | | | | | | | | | | | | | | |
| Brand A | .. | .. | V. sl. stale .. | V. sl. stale .. | V. sl. stale .. | 96 | 97 | 99 | 100 | 97 | 98 | 3·0 | 3·4 | 3·3 |
| Brand B | .. | .. | V. sl. stale .. | V. sl. stale .. | Sl. stale .. | 99 | 98 | 100 | 101 | 97 | 99 | 3·7 | 3·5 | 3·4 |

(1) Blocks stored 12 weeks. (2) Blocks stored 26 weeks. (3) Blocks stored 38 weeks.
I. Sample from interior of block. O. Outside sample to depth of about 5 mm. C. Composite sample.
Cello. = wrapped in heat sealing cellophane. Wax = wrapped in paper and dipped in wax.
Wax + Cello. = Combination of cello. and wax with the cellophane on the inside.

TABLE 4.—KEEPING QUALITY OF COMPRESSED WHOLE MILK POWDER HELD AT ROOM TEMPERATURE AND HUMIDITY.

| Wrapping and Brand. | Increase in Weight of Blocks. | Surface Cracking. | Grading. | | | | Percentage Solubility. | | | | | | | | Moisture. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 1. | | 2. | | 3. | | 4. | | | | | |
| | | | 1. | 2. | 3. | 4. | 20.° | 50.° | 20.° | 50.° | 20.° | 50.° | 20.° | 50.° | 1. | 2. | 3. | 4. |
| | % | | | | | | | | | | | | | | | | | |
| Brand A—Cello. . . | (1) 0·8 | (1) Nil . . | I. Sl. stale | Sl. stale | Sl. stale | Sl. stale | 98 | 99 | 95 | 99 | 95 | 98 | 97 | 99 | 2·9 | 4·0 | 3·7 | 3·8 |
| | (2) 1·2 | (2) Sev. small | O. Sl. stale | Stale . . | V. stale | V. stale, sl. tallowy | 92 | 97 | 68 | 94 | 72 | 86 | 64 | 85 | 3·1 | 6·0 | 5·3 | 5·2 |
| | (3) 1·4 | (3) Sev. fairly deep | C. Sl. stale | Sl. stale | Sl. stale | V. stale, sl. cardb. | 96 | 100 | 90 | 99 | 83 | 94 | 93 | 97 | 3·7 | 4·9 | 4·6 | 4·0 |
| | (4) 1·2 | (4) Sev. fairly deep | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . |
| Wax . . | (1) 0·6 | (1) Nil . . | I. Sl. stale | Sl. stale | Sl. stale | Sl. stale | 97 | 100 | 95 | 98 | 96 | 98 | 95 | 99 | 3·3 | 4·0 | 4·0 | 3·9 |
| | (2) 0·6 | (2) Nil . . | O. Sl. stale | Stale, cardb. | V. stale, cardb. | V. stale, sl. cardb. | 98 | 100 | 95 | 97 | 96 | 99 | 92 | 99 | 3·9 | 5·3 | 4·8 | 5·1 |
| | (3) 0·8 | (3) Nil . . | C. Sl. stale | Sl. stale, sl. cardb. | Sl. stale | Sl. stale | 96 | 101 | 95 | 99 | 94 | 98 | 96 | 95 | 3·4 | 4·3 | 4·3 | 4·2 |
| | (4) 0·8 | (4) Nil . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . |
| Wax + Cello. | (1) 0·0 | (1) Nil . . | I. Sl. stale | V. sl. stale | Sl. stale | Sl. stale | 95 | 98 | 96 | 98 | 97 | 99 | 96 | 96 | 4·3 | 4·0 | 3·8 | 4·1 |
| | (2) 0·4 | (2) Nil . . | O. Sl. stale | Sl. stale | Stale . . | Stale . . | 95 | 99 | 96 | 97 | 96 | 100 | 94 | 98 | 5·3 | 4·4 | 5·0 | 4·9 |
| | (3) 0·6 | (3) Nil . . | C. Sl. stale | V. sl. stale | Stale . . | Sl. stale | 94 | 97 | 96 | 99 | 97 | 100 | 96 | 97 | 4·3 | 3·9 | 4·1 | 4·2 |
| | (4) 0·8 | (4) Nil . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . |
| Brand B—Cello. . . | (1) 0·8 | (1) Nil . . | I. Sl. stale | Sl. stale | Sl. stale | Stale . . | 98 | 100 | 99 | 100 | 99 | 100 | 98 | 99 | 4·0 | 3·5 | 3·4 | 3·4 |
| | (2) 1·4 | (2) Slight . . | O. Sl. stale | Sl. stale | Stale . . | V. stale, sl. cardb. | 97 | 98 | 85 | 98 | 79 | 98 | 76 | 94 | 5·2 | 6·1 | 5·7 | 5·2 |
| | (3) 1·2 | (3) A few mod. deep | C. Sl. stale | V. sl. stale | Stale . . | Sl. stale | 99 | 99 | 96 | 99 | 95 | 99 | 94 | 99 | 3·9 | 4·1 | 4·0 | 4·1 |
| | (4) 1·4 | (4) A few mod. deep | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . | . . |

TABLE 4.—KEEPING QUALITY OF COMPRESSED WHOLE MILK POWDER HELD AT ROOM TEMPERATURE AND HUMIDITY—*continued.*

| Wrapping and Brand. | Increase in Weight of Blocks. | Surface Cracking. | Grading. | | | | Percentage Solubility. | | | | | | | | Moisture. | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1. | 2. | 3. | 4. | 1. | | 2. | | 3. | | 4. | | 1. | 2. | 3. | 4. |
| | | | | | | | 20.° | 50.° | 20.° | 50.° | 20.° | 50.° | 20.° | 50.° | | | | |
| Brand B— | | | | | | | | | | | | | | | | | | |
| Wax .. | (1) 0·6 | (1) Nil .. | I. Sl. stale | Sl. stale | Sl. stale | Sl. stale | 99 | 102 | 100 | 100 | 97 | 94 | 98 | 99 | 3·7 | 3·4 | 3·5 | 3·7 |
| | (2) 0·8 | (2) Nil .. | O. Sl. stale | Sl. stale | Stale .. | Sl. stale, sl. cardb. | 98 | 101 | 100 | 101 | 97 | 101 | 97 | 98 | 4·4 | 5·1 | 5·6 | 5·5 |
| | (3) 1·0 | (3) Nil .. | C. Sl. stale | Sl. stale | Stale .. | Stale .. | 100 | 103 | 99 | 100 | 100 | 100 | 98 | 98 | 4·2 | 3·6 | 3·8 | 4·1 |
| | (4) 0·8 | (4) Nil .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Wax + Cello. | (1) 0·0 | (1) Nil .. | I. Sl. stale | Sl. stale | Stale .. | Stale, sl. cardb. | 95 | 99 | 100 | 99 | 101 | 99 | 98 | 98 | 3·4 | 3·6 | 3·7 | 3·4 |
| | (2) 0·8 | (2) Nil .. | O. Sl. stale, sl. cardb. | Sl. stale | Sl. stale | Stale, sl. cardb. | 96 | 99 | 99 | 99 | 91 | 97 | 96 | 94 | 4·5 | 4·6 | 5·5 | 4·7 |
| | (3) 1·0 | (3) V. sl. sur. and one mod. deep crack | C. V. v. sl. stale | Sl. stale | Sl. stale | Sl. stale sl. cardb. | 98 | 98 | 99 | 99 | 95 | 98 | 95 | 95 | 3·5 | 3·9 | 4·3 | 3·6 |
| | (4) 0·6 | (4) Nil .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| Controls— | | | | | | | | | | | | | | | | | | |
| Brand A | .. | .. | Fresh .. | V. v. sl. stale | Sl. stale | V. sl. stale | 98·7 | 100·1 | 100·7 | 101·7 | 98·0 | 99·3 | 99·4 | 99·2 | 3·22 | 3·55 | 3·46 | 3·50 |
| Brand B | .. | .. | .. | V. sl. stale | .. | V. sl. stale | .. | .. | 99·1 | 99·1 | .. | .. | 99·1 | 99·2 | .. | 3·14 | .. | 3·74 |

(1) Blocks stored 12 weeks. (2) Blocks stored 26 weeks. (3) Blocks stored 38 weeks. (4) Blocks stored 50 weeks.
I. Sample from interior of block. O. Outside sample to depth of about 5 mm. C. Composite sample.
Cello. = wrapped in heat-sealing cellophane. Wax = wrapped in paper and dipped in wax.
Wax + Cello. = Combination of cello. and wax with cellophane on the inside.

## 4. Keeping Quality of Small Blocks and Tablets of Milk Powder containing Cane Sugar and Vanillin.

(i) *Introduction.*

Bulk samples of spray-dried and roller-dried whole milk powder were made into 1-oz. blocks measuring $2\frac{1}{4}$ in. by $1\frac{5}{8}$ in. by 15/32 in., and also into tablets 0·75 in. diameter by 0·34 in. The roller-dried powder contained 5·0 per cent. moisture, 12·5$\mu$g. iron, and 3·6$\mu$g. copper per g., and the spray-dried powder 3·5 per cent. moisture, 2·0$\mu$g. iron, and 0·4$\mu$g. copper per g.

As it was thought possible that tablets of milk powder might be consumed either by eating the dry material or as a liquid after reconstitution, additions were made to some of the tablets. To some, strong vanilla essence at the rate of $\frac{1}{2}$ fluid ounce per 100 lb. of powder was added by spraying the essence on to the powder before compressing; others were made from a mixture of 20 per cent. by weight of table crystals of cane sugar and 80 per cent. milk powder. The latter tablets were much more friable than those made from straight milk powder, and could be readily crushed by hand and reconstituted with warm or even cold water.

The 1-oz. blocks were individually wrapped in waxed paper and heat-sealed. Rouleaux of eight of the round tablets weighing $\frac{3}{4}$ oz. were wrapped and sealed in waxed paper. The various types of tablets were then placed either in wax-sealed tins or in desiccators over saturated KCl solution (approximately 80 per cent. relative humidity) and stored at the following temperatures:—20°C. (68°F.), 30°C. (86°F.), 37°C. (99°F.), and 49°C. (120°F.).

(ii) *Discussion.*

A summary of the results of this experiment is given in Figs. 2 and 3. Generally at any one time the 1-oz. blocks were slightly superior in quality to the small round tablets, but as this difference was slight, separate lines for the two types have not been drawn on the graphs.

Oxidative changes of the fat fraction of the milk powder were not detected at any time during the experiment. Burnt and caramelized flavours occurred in tablets stored at 37°C. and 49°C., particularly under high humidity conditions, but by far the most frequent type of deterioration was "protein staleness," characterized by a gradual loss of fresh milk flavour and subsequently an increase in an off-flavour reminiscent of casein glue. In Figs. 2 and 3 progressive deterioration is indicated by four different types of line. The first detectable change in flavour, which was actually a loss of fullness rather than the appearance of an off-flavour, was taken as the end-point of the first period. The end of the second period was characterized by the appearance of a detectable staleness, and the end of the third period by a definitely stale flavour. At this point the tablets were still quite usable, and the fourth period represents the time taken for the staleness to increase to such a point that the tablets were no longer considered to be edible.

It is apparent from the two graphs that the sealed waxed paper was not an effective barrier to water vapour. At all temperatures the useful life of the tablets was much shorter under high humidity conditions than in the sealed tins. The appearance of mould between the paper and the tablets after four weeks at 37°C. and nine weeks at 30°C. indicated a high relative humidity within the wrapping. It would thus seem necessary to pack compressed milk powder in sealed metal-lined containers, and only depend on such materials as waxed paper for protection in the interval between opening the containers and the consumption of the compressed milk.

The time taken for the tablets to become stale (end of third period) at 49°C. was about one week at 80 per cent. relative humidity and about 33 weeks in sealed tins. At 37°C. the time for the development of a stale flavour was two to three weeks under both conditions, and at 30°C. six to seven weeks at high humidity, and over six months in sealed tins. At 20°C. the tablets were stale under high humidity conditions after about six months, but in sealed tins were still in excellent condition after fourteen months' storage.

Both vanilla essence and sugar added to the milk powder before compressing improved the keeping quality of the tablets. As may be seen from the graphs, the first detectable change did not occur in the tablets to which additions had been made until they had been in store one and a half times to twice as long as the plain milk tablets. At 37° and 49°C. the inedible stage occurred about as quickly with all tablets, but at 20° and 30°C. the additions seemed to increase the total life of the tablets as well as delaying the first stages of deterioration.

Tablets made from roller-dried powder kept about as well as tablets made from spray-dried powder. Also, the temperature coefficients for the first appearance of staleness in compressed roller-dried and spray-dried powders (Table 5) were approximately equal over the temperature ranges used in this experiment. Temperature coefficients were calculated from the data summarized in Figs. 2 and 3 by dividing the storage time for the appearance of staleness at the lower temperature by the storage time at the next highest temperaure and correcting this quotient to a temperature interval of 10°C. Unfortunately, the data are not sufficiently complete to enable temperature coefficients to be calculated for all stages of development of staleness, but it would appear from Table 5 that a coefficient of about 3 could be taken as the figure for first appearance of staleness. This is rather higher than the figure of 2·2 given by Lea *et al.* (1943) for the temperature coefficient of oxidation of butter-fat in whole milk powder.

A few samples of compressed spray-dried whole milk and 20 per cent. cane sugar in the form of 1-lb. blocks have been stored at 30°C. and room temperature. The blocks were packed in tins just large enough to hold five 1-lb. blocks, and the push-on lids were sealed with a ½-in. strip of sticking plaster. After six months at room temperature the blocks showed a very slight heated flavour, and after six months at 30°C. a slight staleness. A further tin of 1-lb. blocks was opened after being held at room temperature for sixteen months. The blocks were then very slightly stale.

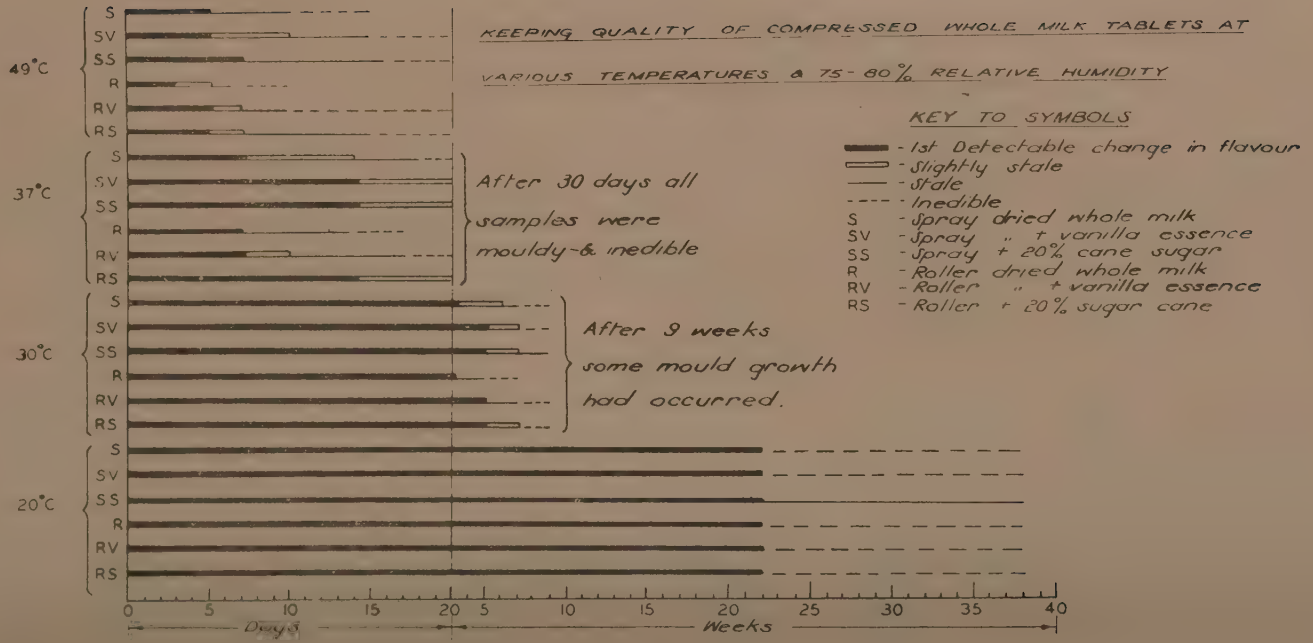
KEEPING QUALITY OF COMPRESSED WHOLE MILK TABLETS AT
VARIOUS TEMPERATURES & 75-80% RELATIVE HUMIDITY
KEY TO SYMBOLS
- 1st Detectable change in flavour
- Slightly stale
- Stale
- Inedible
S - Spray dried whole milk
SV - Spray " + vanilla essence
SS - Spray + 20% cane sugar
R - Roller dried whole milk
RV - Roller " + vanilla essence
RS - Roller + 20% sugar cane
49°C
37°C
30°C
20°C
S
SV
SS
R
RV
RS
After 30 days all samples were mouldy-& inedible
After 9 weeks some mould growth had occurred.
0 5 10 15 20 5 10 15 20 25 30 35 40
Days
Weeks

FIG. 2.

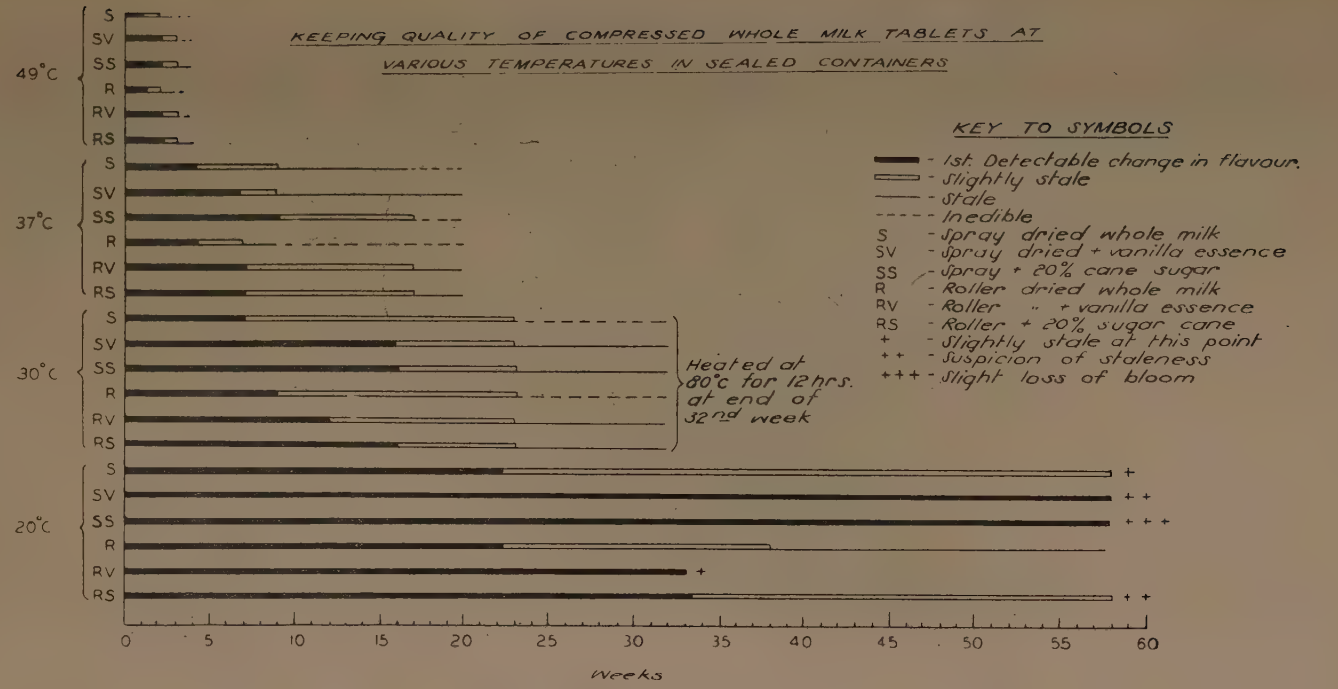
KEEPING QUALITY OF COMPRESSED WHOLE MILK TABLETS AT VARIOUS TEMPERATURES IN SEALED CONTAINERS
49°C
37°C
30°C
20°C
S
SV
SS
R
RV
RS
Heated at 80°C for 12 hrs. at end of 32nd week
KEY TO SYMBOLS
1st. Detectable change in flavour.
Slightly stale
Stale
Inedible
S - Spray dried whole milk
SV - Spray dried + vanilla essence
SS - Spray + 20% cane sugar
R - Roller dried whole milk
RV - Roller " + vanilla essence
RS - Roller + 20% sugar cane
+ - Slightly stale at this point
++ - Suspicion of staleness
+++ - Slight loss of bloom
0 5 10 15 20 25 30 35 40 45 50 55 60
Weeks

FIG. 3.

TABLE 5.—TEMPERATURE COEFFICIENTS FOR FIRST APPEARANCE OF STALENESS IN COMPRESSED MILK TABLETS KEPT IN SEALED CONTAINERS.

| — | 37°–49°C. | 30°–37°C. | 20°–30°C. |
|---|---|---|---|
| S .. .. .. | 3·3 | 2·5 | 3·1 |
| SV .. .. .. | 2·9 | 3·3 | 3·8+ |
| SS .. .. .. | 3·75 | 2·5 | 3·8+ |
| R .. .. .. | 3·3 | 3·2 | 2·5 |
| RV .. .. .. | 2·9 | 2·4 | 2·7 |
| RS .. .. .. | 2·9 | 3·3 | 2·1 |
| Average .. | 3·2 | 2·9 | 3·0+ |

S = plain spray-dried whole milk.
SV = spray-dried milk + vanilla essence.
SS = spray-dried milk + 20 per cent. sugar.
R = plain roller-dried whole milk.
RV = roller-dried milk + vanilla essence.
RS = roller-dried milk + 20 per cent. sugar.

## 5. Acknowledgments.

The author wishes to acknowledge his indebtedness to F. Hart and Co., Melbourne, who permitted, at considerable expense and inconvenience to themselves, much of the work on compression of milk powder to be carried out at their works. The Divisions of Forest Products and Industrial Chemistry of C.S.I.R. also permitted use of their presses, and Dr. W. J. Wiley, G. Loftus Hills, E. G. Pont, and J. Conochie graded the reconstituted samples.

## 6. References.

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Howat, G. R., Smith, J. A. B., Waite, R., and Wright, N. C. (1939).—Factors affecting solubility of milk powders. IV. Influence of speed and duration of stirring on solubility, with a description of a rapid method for solubility determinations. *J. Dairy Res.* **10**: 498.

# Notes on a Method for the Study of Diffusion of Salts through Green Timber.

*By G. N. Christensen, B.Sc.**

*Summary.*

A method of studying the diffusion of water-soluble chemicals through green timber is described; in this method a disc of green wood is interposed as a "membrane" between two compartments of a diffusion cell. A possible technique of preparing and using the cell is set out and the advantages and limitations of the apparatus discussed.

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Many Australian commercial timbers have proved to be refractory to penetration of preservatives by pressure treatments. Consequently, attention has been focused on the alternative method, namely, penetration of green wood by diffusion. In the past the usual method of obtaining quantitative information on this subject has consisted of treating blocks of wood with solutions for given periods, cutting them into sections and analysing each section for the respective salt used.

While information of purely practical significance can be obtained with most certainty by this method, as a means of studying diffusion it has several disadvantages, among which the following may be mentioned:—

(*a*) The natural variation in wood necessitates a number of tests being carried out, i.e., multiple groups of analyses.

(*b*) To obtain reasonable recovery of the chemical, some form of wet or dry combustion, as opposed to leaching, is necessary. This involves preparation of the material in the form of chips, shavings, &c., and introduces the risk of volatilization losses. Apart from any possible reduction in accuracy due to these factors, some preservatives cannot be readily estimated by this method.

(*c*) The diffusion process can be investigated in progress only by the use of matched samples which are removed from treatment after varying periods.

(*d*) In order that the salt may attain sufficient depth of penetration for sampling, e.g., ½ inch., especially in the case of eucalypt truewood, the blocks must remain immersed for relatively long periods of time.

To overcome or reduce these difficulties, a technique has been developed to study diffusion through a wooden disc interposed as a "membrane" in a diffusion cell which is constructed as follows:—

Two glass bottles (A.G.M. L 491 M) 2 in. by 2 in. by 2½ in. in height, and of approximately 110 ml. capacity, have one vertical face ground completely away. When the two openings thus formed are

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* An officer of the Division of Forest Products.

placed together, the bottles form the two compartments of the diffusion cell. The specimen "membrane" or disc is cut to the same shape as, but slightly larger than, the ground off aperture in the side of each bottle. The disc is held in place by two rubber gaskets, cut to overlap the inner and outer edges of the ground off glass wall. When fitted together the whole cell is held firmly by means of screw clamps and metal plates insulated from the glass by rubber pads (see Figs. 1, 2, and 3).

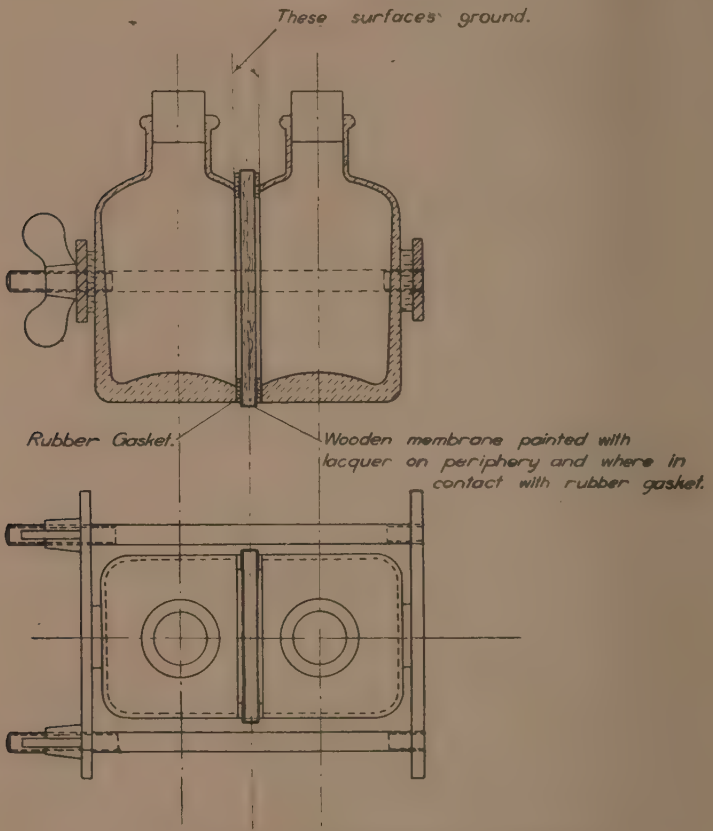


Fig. 1.—Diagrammatic representation of Diffusion Cell.

In setting up the apparatus, the disc is first coated around the periphery and for $\frac{1}{4}$ in. margin around both faces with a double application of lacquer. This has the dual purpose of preventing diffusion of the salt out through the edge of the disc and of forming a water-tight seal with the rubber gasket. Just before setting up the cell, the ground glass faces of the cell are smeared with a trace of petroleum jelly. This helps to prevent passage of the salt solution out between the rubber gasket and the glass. Care is taken to prevent the petroleum jelly from fouling the inside of the cell.

FIG. 2.—Showing diffusion cell set up.

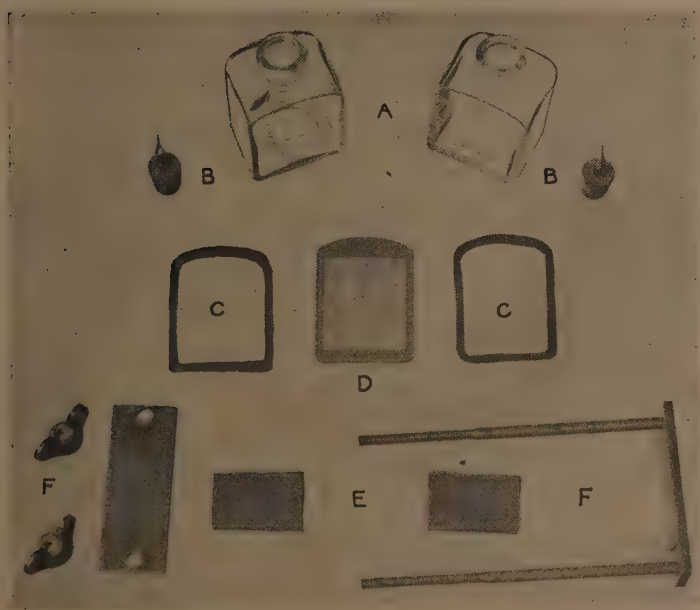


FIG. 3.—Showing component parts of diffusion cell.—A, bottles forming compartments of diffusion cell; B, rubber stopper with capillary; C, rubber gasket; D, lacquered wooden membrane; E, rubber insulating pads; F, clamp system.

Before using the cell, it is preferable to fill both compartments with 100 ml. of distilled water and to leave it for 24 hours at the temperature of the experiment. This serves to extract from the disc sufficient of the water soluble constituents to prevent interference with the analysis. After this preliminary leaching the diffusion test is commenced by emptying the cell and refilling one compartment with distilled water and the other with a solution of the treating salt, each at the appropriate temperature.

To prevent evaporation, both compartments of the cell are stoppered. To avoid unwanted changes in the gaseous pressure in the cell (caused by changes in volume of the solutions due to osmosis), a short open capillary is inserted into each stopper.

At suitable periods (e.g. every 24 hours) the distilled water, into which the salt is diffusing, is removed for analysis. The compartment is then refilled with a fresh 100 ml. of distilled water at the required temperature and left undisturbed for a further period.

In an experiment in which temperature effects were studied, discs of green *Eucalyptus obliqua* truewood (in this case quartersawn) ¼ in. thick were used with 2M sodium chloride solutions. At a temperature of 40°C. the salt diffusing into the distilled water during any 24-hour period attained a concentration of approximately M/100. For most practical purposes one can neglect the change in diffusion rate resulting from the change in concentration due to the movement of this quantity of salt during each 24-hour interval. Under the above conditions a constant rate of diffusion of salt into the distilled water was reached in 6—8 days.

For more accurate work, some form of agitation of the solutions is very desirable, e.g., a rocking or rotating tray. It is also desirable to correct the concentration of the salt solution periodically in order to compensate for:—

(*a*) Increase in volume due to osmosis which can be overcome by evaporation until the volume in the compartment has reached its initial value of 100 ml. This can be done simply by removing the stopper and capilliary.

(*b*) Decrease in concentration as a result of the outward diffusion of the salt which can be adjusted by the addition of a weighed quantity of salt calculated from the progressive analytical results. This correction does not allow for the salt which has diffused into and remained in the wooden disc.

Some advantages of this method of studying diffusion are:—

(*a*) The salt to be analysed is already in solution.

(*b*) Several cells may be employed simultaneously at any desired temperature.

(*c*) The two phases of the diffusion can be studied separately, namely:—

(i) The approach to equilibrium under given conditions can be examined first.

(ii) Once equilibrium has been attained, some of the conditions may be changed, e.g., temperature, increase in concentration, addition of another salt, etc., and the change in diffusion rate measured directly. This obviates the necessity of relying on matched samples.

(*d*) If direct comparison as above is not possible, and matching is necessary, this is more easily achieved with small discs than with larger blocks of wood. Ease of matching will, of course, depend on the direction of the grain desired in the disc.

(*e*) Quantitative results, including analyses, can be obtained in a few days instead of weeks.

The chief advantage of the method lies in the ease of making direct comparisons of the many factors affecting the rate of diffusion of salts *through* wood.

If, on the other hand, the primary objective of the investigation is to obtain quantitative information on the diffusion of chemicals *into* wood (as in preservation processes) evaluation of the diffusion constant and its mathematical application in the light of the known diffusion laws may give the required information. There are, however, several factors present in the disc method which have no counterpart in diffusion into a block of wood:—

(*a*) In so far as wood acts as a semi-permeable membrane, water and salt would diffuse at different rates in opposite directions. In this way, the outside layers of a completely immersed block would act as an osmotic cell with a consequent reduction in pressure inside. There is no such difference in pressure in the diffusion cell since water can move freely.

(*b*) In view of (*a*) and the fact that the volume of water inside a block of wood is limited, the counter diffusion of water through the membrane is of greater magnitude than in the block of similar material.

(*c*) There is a second wood-water interface through which molecules must diffuse. This is not present in a block of wood. Its relative importance would depend on the actual mechanism by which the salts diffuse through the green wood.

It is proposed to use the diffusion cell as a rapid method of studying isolated factors affecting the rate of diffusion of solutes through green timber.

This will involve a study of the effect on diffusion rate of such factors as temperature, concentration and pH of the treating solution, type of salt used, duration of diffusion period, timber species, and direction of the grain.

The ultimate object of the investigation is to apply this information to the development of practical diffusion treatments for the preservation of green timber.

# The Catalytic Dehydration of 2,3-Butanediol to 1,3-Butadiene.*

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*Summary.*

A number of substances have been examined for ability to dehydrate gaseous 2, 3-butanediol through methyl vinyl carbinol to butadiene. In most cases the alternative reaction to methyl ethyl ketone was favoured.

Thorium oxide catalysed the dehydration of the diol to methyl vinyl carbinol, and further to butadiene, under reduced pressure and at temperatures around 350°C. Although the thoria seemed to be specific in its action, some methyl ethyl ketone appeared in the reaction products, owing to dehydration to this compound either in the gas phase, on the walls of the apparatus, or on traces of impurity in the catalyst. The oxide was best prepared by decomposition of pure thorium oxalate at temperatures below 400°C. With thoria as catalyst, single pass conversions of 60 per cent. to butadiene and 80 per cent. to methyl vinyl carbinol plus butadiene have been obtained.

The rate of dehydration to carbinol and butadiene was inversely proportional to the pressure. For one of the thoria catalysts the apparent energy of activation of the reaction in which 2, 3-butanediol was dehydrated to methyl vinyl carbinol was found to be 25,000 calories per mole.

## 1. Introduction.

A direct method is desired for conversion of 2, 3-butanediol to butadiene, to replace the two-stage process of Hill and Isaacs (3) in which diol is esterified to diacetate and the latter pyrolysed at 550-600°C. to yield diene. The present paper describes some attempts to carry out the direct conversion catalytically, thus eliminating the need for acetic acid.

It is well established that 1, 3-butanediol can be dehydrated over a catalyst to 1, 3-butadiene in high yield (5). Attempts to devise an analogous process for the 2, 3-diol generally result in good yields of methyl ethyl ketone and less than 10 per cent. yield of butadiene. The relative difficulty in dehydrating the 2, 3-diol to butadiene is readily understood when its structure is considered. The two hydroxyl groups are on adjacent carbon atoms, facilitating dehydration to the enol form of methyl ethyl ketone. Two additional reactions in which a molecule of water is removed from the 2- and 3-carbon atoms may occur—one to yield 2-butene oxide and another proceeding to methyl ethyl ketone by a pinacolin transformation. In view of these alternative possible reactions it is not surprising that the desired reaction, in which methyl vinyl carbinol and butadiene are successively produced, does not predominate except under special conditions. This is particularly true because the reaction which yields methyl ethyl ketone appears to have a lower activation energy than that which yields methyl vinyl carbinol, as will be shown later.

When designing the experimental methods it was decided to work at low pressures, not entirely because reduced pressure would appear to favour a reaction in which the number of molecules increases, since this consideration is not necessarily valid for a catalytic process, but because it was felt that there was a strong possibility of poisoning of the catalyst by a reaction product.

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* This investigation was carried out in the Chemistry Department of the University of Melbourne.

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## 2. Experimental.

The later measurements of catalytic reaction rates were performed in the apparatus of Fig. 1. The earlier apparatus was essentially the

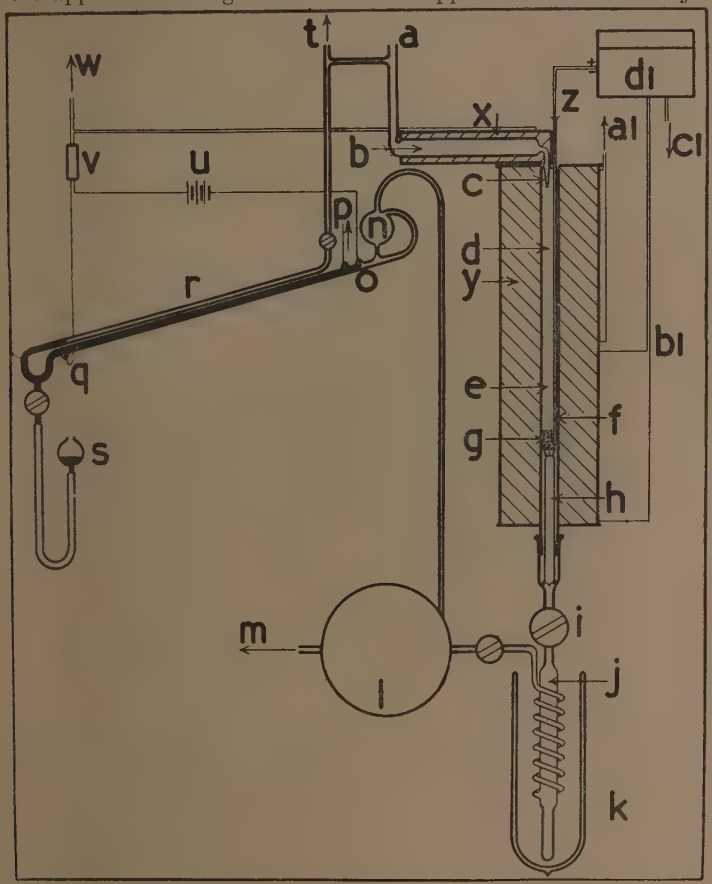


Fig. 1.—Apparatus for Catalytic Dehydration under Reduced Pressure, with Automatic Control of Temperature, Pressure and Flow Rate.

a. Opening for admission of the 5 g. sample of diol to vapourizer b heated by windings x;
c. jet to maintain a pressure gradient between b and preheater d (pyrex 23 mm. internal diameter);
e. catalyst chamber;
f. tube containing thermocouple with leads z to temperature controller $d_1$;
g. catalyst layer 2 cm. deep;
h. sealed tube with upper platform to support the catalyst—the tube hastens removal of gas as soon as it has passed the catalyst;
i. large stopcock;
j. efficient trap cooled with liquid oxygen;
m. to manometer, manostat and pump;
o, q. molybdenum-tungsten contacts to control heater x through relay v;
p. contact to control electric clock;
r. inclined mercury flowmeter to maintain constant flow rate through jet c.

same, with the exception that a horizontal tube 1·3 cm. in diameter was employed, containing a layer of catalyst powder 20 cm. in length. In a few of the earliest experiments the products of dehydration of the 2, 3-butanediol were estimated by separation into a number of fractions by distillation. Later the following simplified analytical procedure was used:—The liquid air-cooled receiver was allowed to warm, and the evolved gas collected over 50 per cent. aqueous glycerol solution, its volume measured, and the percentage of butadiene in the gas determined in the apparatus of Tropsch and Mattox (9) as modified by Kambara (7). Samples of the liquid remaining in the receiver were analysed in duplicate for methyl ethyl ketone (8) by determining the hydrochloric acid liberated from hydroxylamine hydrochloride solution, and for unsaturated compounds by bromine titration. This latter determination was taken as a measure of the amount of methyl vinyl carbinol, an assumption which, although not strictly justifiable,* sufficed for the present investigation.

Contact times were calculated simply from the volume of free space in that length of the catalyst chamber which contained the catalyst, and a knowledge of the time taken to pass 5 g. of 2, 3-butanediol through the system, considering only the volume of gaseous diol and neglecting the volume of products. The 2, 3-butanediol employed was approximately 99 per cent. pure. It was mainly the *l*-isomer, prepared by fermentation of wheat by *Aerobacillus polymyxa*, and 1-butanol extraction of the filtered beer. Its optical activity was $(\alpha)_{15}^{D} - 12{\cdot}8°$, and refractive index $N_{20}^{D}$ 1·432.

Some results of representative runs with a number of different catalysts are shown in Table 1, and the method of preparation of certain of the catalysts in Table 2. Several preliminary runs with

Table 1.—Conversions per Pass to Methyl Vinyl Carbinol, Butadiene, and Methyl Ethyl Ketone Obtained by Catalytic Dehydration of 2, 3-Butanediol Under Reduced Pressure.†

| Run. | Catalyst. | Preheater Temp. | Catalyst Temp. | Press. | Contact Time. | MVC. | Diene. | MEK. | MVC + Diene / MEK. |
|---|---|---|---|---|---|---|---|---|---|
| | | °C. | °C. | mm. | secs. | % | % | % | |
| 12 | Quartz chips | 310 | 480 | 25 | 1·2 | .. | 0 | 15 | 0 |
| 11 | Quartz chips | 300 | 600 | 25 | 1·4 | .. | < 4 | 56 | < 0·07 |
| 16 | C1A, $B_2O_3$ | 335 | 615 | 33 | 0·7 | <7 | 1 | 35 | < 0·2 |
| 24 | C3A, BeO.. | 320 | 500 | 39 | 0·75 | 5·5 | < 5 | 30·5 | < 0·3 |
| 26 | C4, red P on pumice (5) | 280 | 320 | 23 | 0·25 | 0·75 | < 3 | 4·5 | < 0·8 |
| 27 | C4, red P on pumice (5) | 280 | 370 | 40 | 0·4 | 1·5 | < 4 | 8·5 | < 0·6 |
| 29 | C5, $CaHPO_4$ + C(6) | 280 | 350 | 35 | 0·6 | 2·0 | <10 | 8·5 | < 1·4 |
| 28 | C5, $CaHPO_4$ + C(6) | 280 | 400 | 9 | 0·1 | 1·3 | <18 | 54 | < 0·3 |

* Later in the paper it is indicated that 1-hydroxy 2-butene may be present in addition to methyl vinyl carbinol. Both these compounds should, however, yield butadiene on further dehydration.

† See footnote on next page.

TABLE 1.—CONVERSIONS PER PASS TO METHYL VINYL CARBINOL, BUTADIENE, AND METHYL ETHYL KETONE OBTAINED BY CATALYTIC DEHYDRATION OF 2, 3-BUTANEDIOL UNDER REDUCED PRESSURE.†—*continued.*

| Run. | Catalyst. | Preheater Temp. | Catalyst Temp. | Press. | Contact Time. | MVC. | Diene. | MEK. | MVC + Diene / MEK. |
|---|---|---|---|---|---|---|---|---|---|
| | | °C. | °C. | mm. | secs. | % | % | % | |
| 32 | C6, $CH_3COO$ — Na | 280 | 300 | 35 | 3·2 | 0·7 | <7 | 0 | > 1 |
| 35 | C7, $CaCO_3$ | 280 | 550 | 80 | 1·8 | 5·9 | <10 | 54 | < 0·3 |
| 36 | C8, $CaSO_4$ | 280 | 500 | 95 | 2·0 | 12 | < 7 | 71 | < 0·3 |
| 38 | C9, $ThO_2$ .. | 300 | 400 | 60 | 1·9 | 65 | <12 | 20 | > 3·5 |
| 40 | C9A, $ThO_2$ | 280 | 300 | 90 | 2·6 | 14 | 0 | 6·5 | 2·1 |
| 41 | C9A, $ThO_2$ | 280 | 350 | 60 | 1·8 | 45 | 0 | 15 | 3·0 |
| 47 | C9A, $ThO_2$ | 280 | 350 | 10 | 1·7 | 35 | 29 | 14 | 4·5 |
| 46 | C9A, $ThO_2$ | 280 | 400 | 42 | 1·8 | 6·7 | 43 | 17 | 2·9 |
| 44 | C9A, $ThO_2$ | 280 | 400 | 95 | 2·3 | 32 | 23 | 28 | 1·9 |
| 48 | C9A, $ThO_2$ | 280 | 400 | 140 | 3·3 | 31 | 25 | 16 | 3·5 |
| 49 | C9A, $ThO_2$ | 280 | 400 | 250 | 4·6 | 36 | 13 | 29 | 1·6 |
| 50 | C9A, $ThO_2$ | 300 | 500 | 10 | 0·32 | 26·9 | 41·2 | 17·9 | 3·8 |
| 53 | C9A, $ThO_2$ | 300 | 500 | 70 | 1·2 | 6·2 | 57 | 19·9 | 3·1 |
| 52 | C9A, $ThO_2$ | 300 | 500 | 70 | 1·4 | 8·4 | 62·1 | 26·2 | 2·7 |
| 43 | C9A, $ThO_2$ | 280 | 550 | 95 | 1·7 | 14 | 19 | 35 | 0·9 |
| 76 | C11, $ThO_2$.. | 280 | 400 | 16 | 0·56 | 11·6 | 4·4 | 33·5 | 0·47 |
| 77 | C11, $ThO_2$.. | 280 | 450 | 16 | 0·53 | 19·6 | 11·7 | 28·5 | 1·1 |
| 79 | C12, $ThO_2$.. | 280 | 450 | 16 | 0·55 | 31·5 | 6·8 | 36·3 | 1·0 |
| 84 | C14, $ThO_2$.. | 280 | 450 | 16 | 0·56 | 44·8 | 5·1 | 23·9 | 2·0 |
| 85 | C15, $ThO_2$.. | 280 | 450 | 16 | 0·57 | 57·6 | 4·6 | 21·6 | 2·8 |
| 88 | C16, $ThO_2$.. | 280 | 450 | 16 | 0·67 | 34·5 | 22·4 | 16·1 | 3·5 |
| 92 | C17, $ThO_2$.. | 280 | 450 | 16 | 0·72 | 15·5 | 10·1 | 29·8 | 0·85 |
| 96 | C18, $ThO_2$.. | 280 | 450 | 16 | 0·67 | 15·8 | 21·4 | 27·2 | 1·3 |
| 98 | C19, $ThO_2$.. | 280 | 400 | 16 | 0·72 | 16·9 | 17·4 | 31·9 | 1·0 |
| 100 | C21, $ThO_2$.. | 280 | 450 | 16 | 0·42 | 16·6 | 40·9 | 14·9 | 3·8 |
| 102 | C23, C24 on pumice | 280 | 450 | 16 | 0·17 | 21·7 | 12·0 | 39·5 | 0·85 |
| 118 | C25, $ThO_2$.. | 280 | 350 | 64 | 1·3 | 52·6 | 4·6 | 6·1 | 9·4 |
| 119 | C25, $ThO_2$.. | 280 | 350 | 64 | 1·6 | 59·7 | 8·5 | 6·6 | 10 |
| 120 | C25, $ThO_2$.. | 280 | 350 | 64 | 2·1 | 70·3 | 3·5 | 7·6 | 9·7 |
| 121 | C25, $ThO_2$.. | 280 | 375 | 64 | 0·81 | 55·2 | 8·8 | 7·8 | 8·2 |
| 122 | C25, $ThO_2$.. | 280 | 375 | 64 | 0·94 | 55·7 | 15·6 | 6·8 | 10 |
| 124 | C25, $ThO_2$.. | 280 | 375 | 64 | 1·2 | 66·0 | 16·1 | 8·3 | 9·9 |
| 125 | C25, $ThO_2$.. | 280 | 375 | 64 | 1·6 | 65·7 | 20·9 | 9·0 | 9·6 |
| 127 | C25, $ThO_2$.. | 280 | 375 | 64 | 2·2 | 64·3 | 22·6 | 11·6 | 7·4 |
| 129 | C25, $ThO_2$.. | 280 | 400 | 64 | 0·69 | 51·2 | 22·2 | 9·6 | 7·6 |
| 131 | C25, $ThO_2$.. | 280 | 400 | 64 | 0·87 | 56·9 | 21·4 | 10·4 | 7·5 |
| 132 | C25, $ThO_2$.. | 280 | 400 | 64 | 1·0 | 57·1 | 24·6 | 9·1 | 8·9 |
| 133 | C25, $ThO_2$.. | 280 | 400 | 64 | 1·2 | 57·6 | 27·2 | 10·3 | 8·2 |
| 134 | C25, $ThO_2$.. | 280 | 400 | 64 | 1·55 | 66·0 | 20·8 | 11·1 | 7·8 |
| 135 | C25, $ThO_2$.. | 280 | 400 | 64 | 2·1 | 51·4 | 30·4 | 14·4 | 5·6 |
| 136 | C25, $ThO_2$.. | 280 | 400 | 64 | 1·25 | 62·0 | 25·1 | 9·4 | 9·2 |
| 138 | C25, $ThO_2$.. | 280 | 425 | 64 | 0·84 | 37·2 | 35·6 | 13·9 | 5·2 |
| 139 | C25, $ThO_2$.. | 280 | 425 | 64 | 1·01 | 35·2 | 42·5 | 14·8 | 5·2 |
| 140 | C25, $ThO_2$.. | 280 | 425 | 64 | 1·20 | 39·9 | 42·6 | 18·2 | 4·5 |
| 141 | C25, $ThO_2$.. | 280 | 425 | 64 | 1·53 | 29·1 | 52·8 | 18·5 | 4·4 |
| 142 | C25, $ThO_2$.. | 280 | 425 | 64 | 2·06 | 26·5 | 48·6 | 22·2 | 3·4 |
| 143 | C25, $ThO_2$.. | 280 | 425 | 64 | 1·00 | 28·9 | 53·7 | 17·1 | 4·5 |
| 144 | C25, $ThO_2$.. | 280 | 450 | 64 | 0·58 | 16·9 | 49·5 | 19·8 | 3·3 |
| 147 | C25, $ThO_2$.. | 280 | 450 | 64 | 0·92 | 32·5 | 43·3 | 23·2 | 3·2 |
| 148 | C25, $ThO_2$.. | 280 | 450 | 64 | 1·11 | 30·3 | 42·7 | 27·5 | 2·6 |
| 149 | C25, $ThO_2$.. | 280 | 450 | 64 | 1·42 | 26·6 | 31·1 | 22·5 | 2·5 |

† All the runs in the above table were conducted in a horizontal catalyst chamber. The conversions are expressed as percentages of the theoretical maximum. In run 16 there was a 10 per cent. yield of a liquid boiling at 140°C., and a 20 per cent. yield of a liquid boiling at 200–250°C.

TABLE 2.—PREPARATION OF THORIA CATALYSTS.

| Catalyst. | Source. | Precipitant. | Number of Times Washed. | Dried at 150°C. | Subsequent Treatment. |
|---|---|---|---|---|---|
| C. 11 .. | Nitrate, Schuchardt | .. | .. | .. | 270°C. (90 mins.), 400(70), 450(10) |
| C. 15 .. | Nitrate, Brit. D. Houses | .. | .. | .. | 800(50), ground 400 (60)*a* |
| C. 12 .. | Nitrate, Merck | $NH_4OH$ aq. | 2 | + | 600(150) |
| C. 14 .. | Nitrate, Brit. D. Houses | $NH_4OH$ aq. | 6 | + | 800(30), ground |
| C. 16 .. | Nitrate Brit. D. Houses | $NH_4OH$ aq. | 7 | + | ground, 400(30), 450(45)*a* |
| C. 17 .. | Sulphate, Brit. D. Houses | $NH_4OH$ aq.*c* | .. | + | ground, 370(30), 450(40) |
| C. 19 .. | Sulphate, Brit. D. Houses | $NH_4OH$ aq.*c* | .. | + | ground,*d* 300–350(120), 450(150) |
| C. 21 .. | Nitrate, Brit. D. Houses | Oxalic acid | 4 | + | 350–400(120), 550(10)*a* |
| C. 24 .. | Nitrate, Brit. D. Houses | Oxalic acid*e* | 10 | + | 350–400(480), 450(30)*a* |
| C. 25 .. | Nitrate, Brit. D. Houses | Oxalic acid*e* | 10 | + | 350–400(360), 350(120)*b* |
| C. 26 .. | Acetate + $SiO_2$ gel.*f* | .. | .. | + | 350(180) |
| C. 18 .. | C. 17 .. | .. | .. | .. | Ground finely, 800(10) |

*a*. In a stream of nitrogen.
*b*. In vacuum.
*c*. Double precipitation.
*d*. Then washed with 1 per cent. acetic acid, followed by water 3 times, drying and grinding.
*e*. Containing 1 per cent. $HNO_3$; the precipitate was allowed to stand 2 days before washing.
*f*. Dialysed, dried, and then soaked in the thorium acetate solution.

quartz chips in place of catalyst were conducted in order to obtain a measure of the degree of thermal decomposition of the diol to be expected at high temperatures. The extent of reaction proved to be small up to 400° C., but 20 per cent. or more above 500 C., the principal product being methyl ethyl ketone.

C.1. In the presence of boric acid or oxide, dehydration of diol to methyl ethyl ketone was suppressed to some extent; however, there was no appreciable yield of methyl vinyl carbinol or butadiene. There was a tendency to remove water by condensing two or more molecules of the diol, yielding high boiling products.†

C.2. Alumina containing 16 per cent. sodium dihydrogen phosphate proved to be a highly active dehydrating catalyst. More than 80 per cent. of the theoretical yield of methyl ethyl ketone was obtained, but little methyl vinyl carbinol or butadiene.

† In some unpublished experiments it was found that vacuum distillation of a mixture of boric acid and 2, 3-butanediol, even in the presence of water, yielded several boric esters, the relative amounts of which depended partly on the temperature at which the distillation was carried out and the water concentration. The esters were hydrolysed when shaken with water, yielding oily liquids, some of which are yet to be identified. Among the products of this boric acid catalysed dehydration are eight- and twelve-carbon straight-chain and cyclic alcohols and diols.

C.3 and C.3A. Beryllium oxide was found to yield much greater amounts of unsaturated compounds than alumina or its mixtures. Much of the product was probably methyl vinyl carbinol, as indicated by the boiling points of the fractions obtained by distillation. More than 50 per cent. of the dehydration was still in the direction of ketone, however.

C.4 and C.5. These catalysts were prepared according to the methods of Johnson and Johnson (5, 6) who claimed for them very good yields of butadiene from the 1, 3-butanediol. It was necessary to employ relatively low temperatures* in the runs with C.4 in view of the reputed instability of red phosphorus above 300°C. A little carbinol and diene was obtained, but with both catalysts the reaction to ketone predominated.

C.6, C.7, C.8. When the hydroxyl groups of 2, 3-butanediol are esterified with a carboxylic acid, the resultant ester can be pyrolysed largely to methyl vinyl carbinol ester, and thence to butadiene. A catalyst whose dehydrating mechanism can be pictured as *via* an ester type of intermediate might therefore be expected to favour dehydration of the diol to carbinol. Sodium acetate, calcium carbonate, and calcium sulphate were used as catalysts with this in mind.

C.9 and C.9A. A commercial thorium oxide was found to favour dehydration to carbinol and diene at the expense of the reaction to ketone. By varying the conditions of temperature, pressure, and flow rate, it was shown that the rate of dehydration increased with decreasing pressure, and that a conversion greater than 50 per cent. of theoretical to butadiene was attainable in one pass. The ketone conversion (15-20 per cent. of theoretical) was not yet as low as desired, however, and so the influence of preparative method upon the catalytic activity of thoria was investigated. It seemed possible that pure thoria might selectively catalyse the dehydrations to methyl vinyl carbinol and butadiene, while most other substances present as impurities would catalyse the reaction to ketone, thus reducing the yield of the two former compounds. Spectrographic analysis† of C.9 and C.9A for suspected impurities revealed only the following:—Mg, Al, Ca, Fe, 0·01 per cent.; Sc, Zr, Ce, not detectable.

C.11 and C.15. Thoria prepared by ignition of the nitrate proved an unsatisfactory catalyst owing to the presence of traces of nitrate. Longer heating at high temperatures would overcome this difficulty, but it was expected that prolonged heating would result in sintering. With later catalysts this was found to be the case when temperatures of 450°C. or more were employed for long periods.

C.12, C.14, C.16, C.17, C.19. Thoria prepared from a salt *via* the hydroxide was likewise unsatisfactory. The hydroxide precipitates were difficult to wash and the process of dehydration of the gels to oxide tedious. The oxide was obtained in the form of dense granules, whose catalytic activity with regard to formation of carbinol and diene was poor and could not be improved by grinding to a fine powder.

* Temperatures approximately 100°C. higher are required for dehydration of the 2, 3-diol as compared with the 1, 3-isomer.

† Carried out by Mr. R. J. Goldacre.

Yields increased with the number of times the gels were washed, showing that anions such as nitrate and sulphate either catalyse ketone formation or inhibit the formation of carbinol and diene. Little improvement of the catalysts could be effected by heating in the temperature range 450-600°C.

C.18. A sample of C.17 was ground and then heated for a short time at 800°C. in the expectation that some sulphate would be removed. The ketone yield was thus appreciably reduced.

C.25. A 10 per cent. excess of hot oxalic acid solution containing 1 per cent. of $HNO_3$ was dropped very slowly into a hot solution of 25 g. of thorium nitrate in 1 l. of water stirred as vigorously as possible. Stirring was continued for a further fifteen minutes and then the mixture allowed to stand for two days. Thorium oxalate prepared in this manner was readily washed free from nitrate. The precipitate was washed with 800 ml. of distilled water ten times by stirring followed by centrifugation, then dried at 150°C. The product was ashed at 350°-400°C. for six hours and heated under vacuum in the catalyst chamber for two hours at 350°C. before use. C.25 proved to be the most effective catalyst for dehydration to carbinol and diene employed in the present investigations. A combined conversion to the two latter compounds of more than 80 per cent. of the theoretical maximum could be attained in one pass. A cumulative butadiene yield of 90 per cent. should be possible by operating at high flow rates and recycling the methyl vinyl carbinol and unchanged diol.

C.23 and C.23A. Pumice was found to be an unsuitable carrier for thoria. The same conclusion was reached for stainless steel gauze (C.27) and silica gel (C.26). In the latter case considerable polymerization of butadiene occurred, possibly as a result of slow desorption of the diene from the interior of the catalyst granules.

C.28. Thoria identical with that of C.9A was compressed into pellets 3 mm. in diameter. A layer 2 cm. deep in the apparatus of Fig. 1 was employed to study the reaction 2, 3-butanediol $\rightarrow$ methyl vinyl carbinol + water as a function of pressure, temperature, and flow rate. In order to reduce to negligible proportions the conversion to butadiene, high rates of flow were employed; at the higher temperatures and lower flow rates, however, small amounts of butadiene inevitably appeared, causing some error in the values for methyl vinyl carbinol yields. Results which did not fit exactly to a smooth curve were thought to be due to variations in the extent of methyl ethyl ketone formation either in the homogeneous gas phase or on the glass walls of the apparatus.

The weight of catalyst C.28 was 21·3 g., its volume in benzene 2·4 cc., and its surface area 5·1 sq. m. per g. as determined by the method of Brunauer, Emmett, and Teller (2).

### 3. Discussion.

Some progress towards understanding the mechanism of dehydration of 2, 3-butanediol over thoria has been achieved by kinetic treatment of the experimental measurements in Table 1. The increase in conversion to ketone with decreasing flow rate normally obtained with

relatively pure thoria is shown in Fig. 2. It will be seen in Figs. 2, 3, and 4 that, following the maximum in the curve for total carbinol (percentage carbinol + percentage diene) production, there is a decrease (more or less rapid according to the temperature) caused by increased formation of ketone and polymerization of diene.

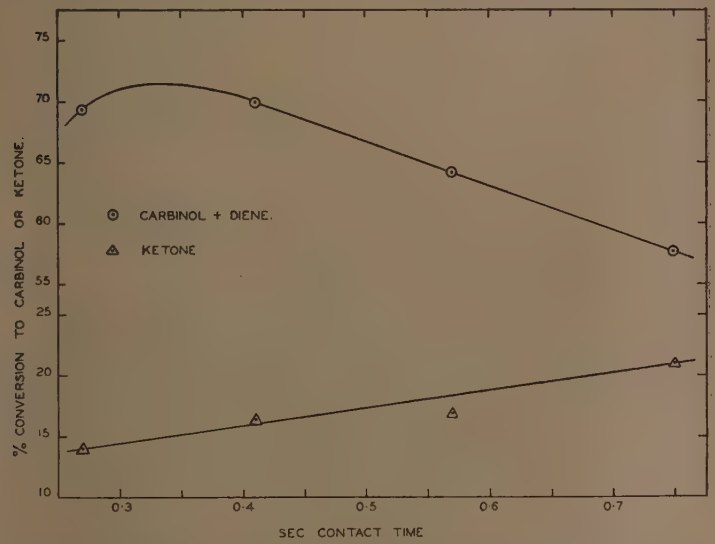


FIG. 2.—Conversions to methyl vinyl carbinol, butadiene, and methyl ethyl ketone during catalytic dehydration of 2, 3-butanediol at 450° C. and 16 mm. pressure. Thoria catalyst C.9A, runs 55–58.

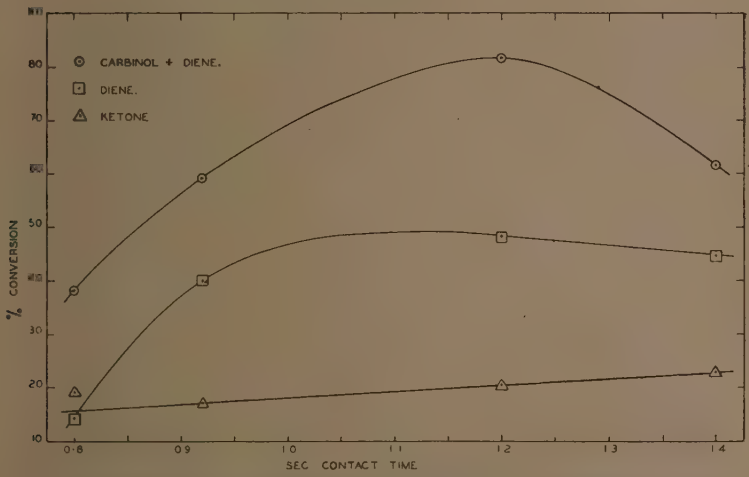


FIG. 3.—Dehydration of 2,3-butanediol at 456°C. and 64 mm. pressure. Thoria catalyst C.24, runs 104–7.

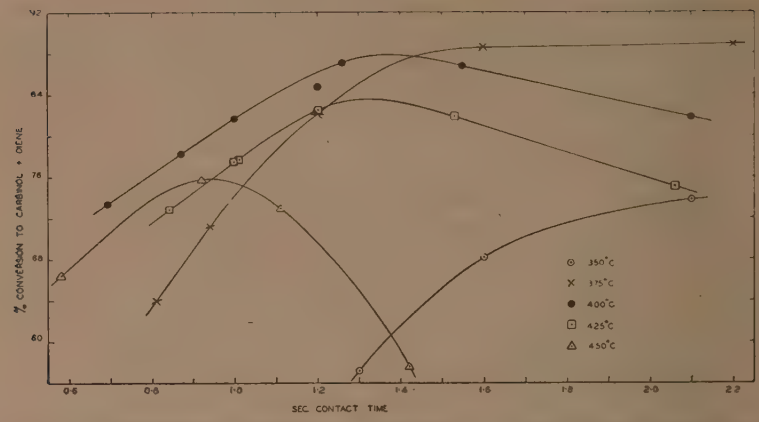


FIG. 4.—Temperature dependence of conversions to methyl vinyl carbinol and butadiene at 64 mm. pressure. Thoria catalyst C.25, runs 118–149.

Conversions for catalyst C.24 to ketone, total carbinol, and diene as functions of flow rate at 64 mm. pressure and 450°C. are presented in Fig. 3. These curves are typical for a fresh sample of fairly pure thoria. It will be noticed that the curve in Fig. 4 representing total conversions to carbinol for C.25 at 450°C. has a lower maximum than the corresponding curve in Fig. 3, probably the result of deterioration of the catalyst after long heating.*

Fig. 4 shows the effect of temperature on the shape of the conversion-flow rate curve for total carbinol produced over catalyst C.25 at 64 mm. pressure. At temperatures up to 375°C. there is a plateau permitting considerable latitude in the flow-rate necessary for maximum conversion in one pass. Consideration of Fig. 4 and the corresponding conversions to ketone in Table 1 reveals that the maximum cumulative yield of carbinol (including carbinol dehydrated further to diene) will be attained at high flow rates and a temperature of about 350°C. (when the pressure is in the neighbourhood of 64 mm.). In one case a cumulative carbinol yield of 90 per cent. was attained.

The earlier experiments, with a long layer of catalyst in a horizontal tube, indicated that the extent of the dehydration to carbinol decreased in proportion to some power of the total pressure $p$. More careful measurements with catalyst C.28 in the apparatus of Fig. 1 showed that for this catalyst at 350°C.

$$y = \frac{c}{p} + b$$

where $y$ is the conversion to carbinol, and $c$ and $b$ are constants (Fig. 5). The process, diol $\xrightarrow{\text{C.28}}$ carbinol + water, may be considered therefore to be a unimolecular reaction in which the rate controlling step is the desorption of one of the products from the catalyst surface (4); until adsorption and desorption measurements are carried out it will be

* When fresh, C.25 was slightly more active than C.24. A number of other thoria catalysts were observed to lose activity in successive steps rather than smoothly, the process being moderately slow at 450°C. and quite rapid at 500°C.

assumed that desorption of water is the slow step, because water is difficult to remove from thoria even by prolonged heating at temperatures above 500°C. If carbinol desorption were slow, the carbinol might be expected to dehydrate almost completely to butadiene, particularly at the lower temperatures, whereas the reverse was found to be true.

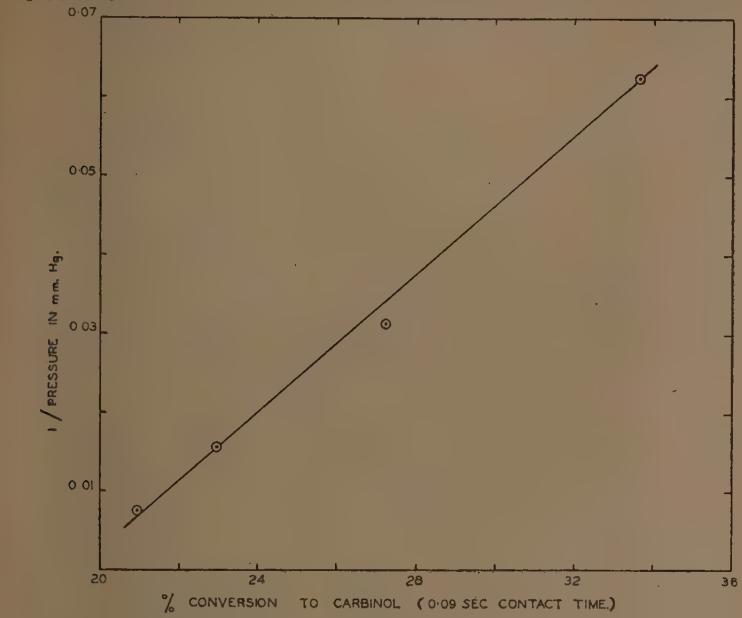


FIG. 5.—Pressure dependence of dehydration of 2, 3-butanediol to methyl vinyl carbinol at 350°C. Thoria catalyst C.28, runs 156–174.

If experiments be continued at lower and lower pressures, a pressure will be found at which desorption of water is no longer limiting, but instead the adsorption of diol. It is in this region that the maximum yields of butadiene will be attained.

Temperature dependence of the dehydration to carbinol over catalyst C.28 at 64 mm. pressure was determined by several runs at various flow rates at each of a series of temperatures. Values of the apparent rate constant ($k_{app}$) for the given catalyst were calculated from the equation (4)—

$$\frac{dx}{dt} = k_{app} \frac{(a' - x)}{x} \quad \ldots\ldots \quad (i)$$

where $a'$ is the initial diol concentration in mols. per l. and $x$ is the carbinol concentration at time $t$ secs. When plotted according to the simple Arrhenius equation

$$k_{app} = a.e^{\frac{-E}{RT}}$$

the points conformed sufficiently well to a straight line (Fig. 6) to permit calculation of the apparent energy of activation $E_{app}$ and the frequency factor $a$, which were found to be 25,000 cal. mole$^{-1}$ and $3 \times 10^8$ mole litre$^{-1}$ sec.$^{-1}$ respectively.

No reliable value for the energy of activation of the dehydration to ketone could be calculated because of the uncertain extent to which this reaction occurred on the catalyst itself. When the reaction was

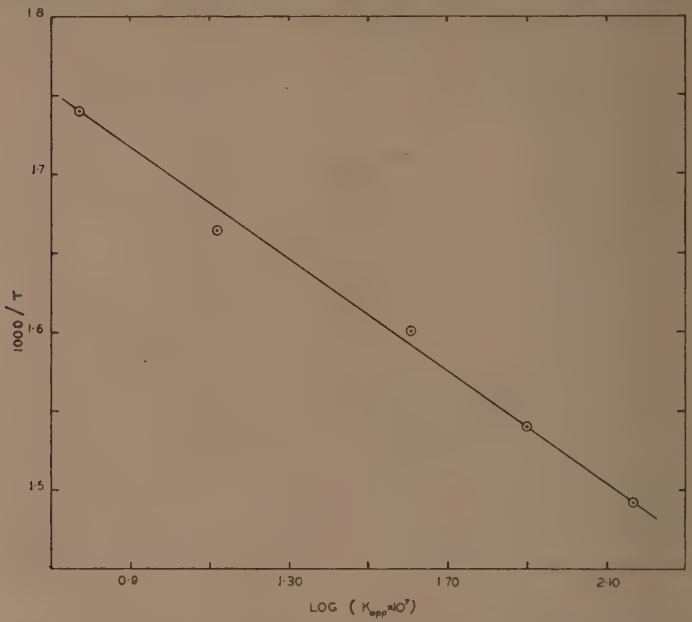


Fig. 6.—Influence of temperature on rate of dehydration of 2, 3–butanediol to methyl vinyl carbinol at 64 mm. pressure. Thoria catalyst C.28, runs 176–209.

assumed to occur entirely on the catalyst, and according to equation (i) (where $x$ is now the concentration of ketone at time $t$) $E_{app}$ and $a$ were calculated to be 16,000 cals. and 0·03 respectively. The much lower energy of activation for the dehydration to ketone* explains why it is difficult to obtain high yields of carbinol and diene. In order to reduce the ketone yield to, say, 5 per cent. of the carbinol yield, it will be necessary to preheat the diol at the minimum temperature in a chemically inert vessel, and to operate with a catalyst free of even minute areas on which the reaction to ketone can occur. It may also be possible to find a catalyst on which the reaction which produces carbinol has an activation energy appreciably less than 25,000 cals.

Table 3 contains the approximate values for the free energy changes and equilibrium constants at one atmosphere pressure for eleven of the reactions which are of interest in the dehydration of 2, 3-butanediol. The energy functions were calculated from the group contributions of Andersen, Beyer, and Watson (1). It will be noticed that at 500°C. the equilibrium constant for the dehydration to ketone is $10^5$ greater than that for the reaction to carbinol. The calculations suggest that

* In the case of catalyst C.25, $E_{app}$ for ketone formation was calculated to be at least 4,000 cals. lower than $E_{app}$ for the production of carbinol.

1-hydroxy-2-butene could be a product alternative to carbinol as an intermediate in diene formation; however, they are not sufficiently accurate to indicate which of these is the most probable intermediate.

Table 3.

| Reaction. | 25°C. | | 500°C. | |
|---|---|---|---|---|
| | $\triangle G°$ cal. | K | $\triangle G°$ cal. | K |
| diol —> ketone + $H_2O$ .. | −18,000 | $6 \times 10^{12}$ | −34,000 | $3 \times 10^{9}$ |
| diol —> diene + $2H_2O$ .. | + 800 | 0·3 | −28,000 | $9 \times 10^{7}$ |
| diol —> 2—OH—2—butene + $H_2O$ | − 2,900 | $1·3 \times 10^{2}$ | −20,000 | $4 \times 10^{5}$ |
| diol —> carbinol + $H_2O$ .. | + 600 | 0·3 | −17,000 | $8 \times 10^{4}$ |
| diol —> 1—OH—2—butene + $H_2O$ | + 120 | 0·8 | −15,000 | $1 \times 10^{4}$ |
| 1—OH—2—butene —> diene + $H_2O$ .. .. .. | + 700 | 0·3 | −14,000 | $8 \times 10^{3}$ |
| carbinol —> diene + $H_2O$ .. | + 160 | 0·8 | −11,000 | $1 \times 10^{3}$ |
| 2—OH—2—butene —> diene + $H_2O$ .. .. .. .. | + 3,700 | $2 \times 10^{-3}$ | − 9,000 | $3 \times 10^{2}$ |
| diene —> ½ dimer .. .. | −13,000 | $2 \times 10^{9}$ | − 3,200 | 8 |
| carbinol —> 2—OH—2—butene .. | − 3,500 | $4 \times 10^{2}$ | − 2,400 | 5 |
| ketone —> diene + $H_2O$ .. | +18,000 | $4 \times 10^{-14}$ | + 5,000 | $4 \times 10^{-2}$ |

## 4. Acknowledgments.

The investigation was carried out in collaboration with Associate Professor W. Davies of the University of Melbourne.

It is desired to acknowledge the frequent advice of Dr. I. W. Wark and Dr. H. H. Hatt, who suggested the problem, and to thank Mr. W. G. Crewther, who supplied the cultures of *Aerobacillus polymyxa*.

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# Mineral Chlorination Studies.

## 2. The Production of Phosphorus Oxychloride by Direct Chlorination of Phosphate Rock.

*By F. K. McTaggart, M.Sc.**

*Summary.*

A process for the manufacture of phosphorus oxychloride has been described which, although not able to compete in cost with the usual methods, afforded a simple means of producing a quantity of this material for essential purposes at a time when supplies were unobtainable elsewhere.

### 1. Introduction.

The chlorination of various types of mineral phosphates for the direct production of phosphorus oxychloride has been the subject of numerous patents which date back to 1920. There is, however, little evidence that any of the suggested processes have been used on an industrial scale. Where alternative sources of phosphorus oxychloride are available there may be little to recommend the method, although in certain special instances it affords a convenient means for the simultaneous production of anhydrous metallic chlorides and phosphorus oxychloride. Using the method described in this paper, phosphorus oxychloride has been produced on a pilot-plant scale from bone ash and from phosphorite from Ocean Island, and was used in Australia for the production of essential drugs during the present war when overseas supplies were unavailable. Although the use of this method must be regarded as a special war-time measure, it was considered desirable to place on record an account of its development.

### 2. The Reaction.

The reaction may be given in two general forms thus:

$$XPO_4 + 3COCl_2 = POCl_3 + XCl_3 + 3CO_2 \quad .. \quad (I.) \text{ or}$$
$$XPO_4 + 3C + 3Cl_2 = POCl_3 + XCl_3 + 3CO \quad .. \quad (II.)$$

The patent literature (1) refers more particularly to the mechanical methods involved in the process rather than to the underlying reaction. Suggested methods involve the following:—

The use of a rotary kiln in which the phosphatic mixture, in powder form, is continually stirred up. The use of continuous mechanical agitation during chlorination by, for example, grinding or stirring. The regeneration of the charge after partial chlorination by leaching out the involatile chlorides formed (e.g. $CaCl_2$). The use of the phosphate of a metal which forms a readily fusible chloride (e.g. $Zn_3(PO_4)_2$, from the reaction—

$$3ZnCl_2 + 2Na_3PO_4 \longrightarrow Zn_3(PO_4)_2 + 6NaCl)$$

so that the chloride formed during reaction drains off to the lower end of the furnace leaving fresh phosphate exposed. It will be noted that the main idea in all these processes is to ensure complete reaction of the phosphate by avoiding clogging of the charge due to reaction products other than $POCl_3$, and to expose a continual fresh surface of the raw material.

* An officer of the Division of Industrial Chemistry.

Most of the patents mentioned phosgene as the chlorinating agent, or else a mixture of CO and $Cl_2$, which at the temperature of the reaction amounts to the same thing. Some also suggested that chlorine could be used provided that the charge was intimately mixed with activated carbon, in which case it was stated that a slightly higher temperature was necessary. The carbon, it was claimed, could be activated by pre-treatment with chlorine at 1000°C. followed by washing and drying before mixing with the phosphate. Chlorination temperatures of from 300° to 600°C. were quoted for various phosphates, and all patents claimed yields of 90–100 per cent. based on the phosphatic content of the raw material. In no case was a figure given for the efficiency based on the consumption of chlorinating agent.

In only one process, that in which the phosphate of a metal having a readily fusible chloride was used, was the charge moulded into pellets (apparently non-porous) before chlorination. The chloride, fused as formed, was expected to drain from the remaining phosphate which was then further attacked by the gas.

It was anticipated that the difficulty of clogging and covering of the charge could be overcome by the use of porous briquettes, into which the chlorinating agent could penetrate, replacing the phosphate by chloride. Furthermore, because of the obvious disadvantages of using the poisonous gas phosgene, or a mixture of carbon monoxide and chlorine which would require careful regulation for highest efficiency, the project aimed at the direct chlorination, by chlorine, of calcium phosphate of the type occurring at Ocean Island and Nauru Island, this material being moulded into briquettes together with carbon. The reaction involved is essentially—

$$Ca_3(PO_4)_2 + 6C + 6Cl_2 \longrightarrow 2POCl_3 + 3CaCl_2 + 6CO \quad \text{(III.)}$$

Weight ratios—

$$4{\cdot}0 + 0{\cdot}92 + 5{\cdot}46 \longrightarrow 4{\cdot}0 + 4{\cdot}27 + 2{\cdot}16$$

However, it was found that some carbon dioxide was formed during the reaction and therefore less carbon was required than the theoretical amount. Actually a ratio of six parts of phosphate to one of carbon was effective, whereas the above equation requires a ratio of 4:1. Excess carbon has no undesirable effects: it is, in fact, beneficial. Naturally occurring phosphates are complex in nature and may be of the fluor-, chlor-, or hydroxy-, apatite type in which there is one atom of fluorine, or chlorine, or one molecule of water for every 3 or 4 phosphorus atoms. As a consequence neither the theoretical yield of the oxychloride, nor the indicated chlorine efficiency of 50 per cent. are obtained. Thus it was impossible to predict the yield accurately without a knowledge of the exact composition of the raw material and the exact mechanism of the reaction.

### 3. Raw Materials.

(i) *Precipitated Calcium Phosphate Prepared from Bones.*

This was a white powder, 100 per cent. minus 350 mesh and was the purest form of calcium phosphate obtainable. It contained combined moisture, i.e., it was a hydroxy type of phosphate.

(ii) *Phosphate from Ocean Island.*

Ocean Island phosphate was chosen because it has the highest phosphate content of any of the naturally occurring phosphates available to Australia and because adequate stocks were held. The material used in the course of this investigation was light grey in colour and had the composition indicated in Table 1.

TABLE 1.

| Component. | Per cent. Before Ignition. | Per cent. After Ignition to 650°C. |
|---|---|---|
| Moisture .. .. .. .. | 3·4 | .. |
| $P_2O_5$ .. .. .. .. | (40·2) | (42·3) |
| $Ca_3(PO_4)_2$ .. .. .. .. | 87·7 | 92·4 |
| $CaCO_3$ .. .. .. .. | 3·4 | 3·6 |
| $Fe_2O_3$ &c. .. .. .. .. | 0·7 | 0·8 |
| Organic .. .. .. .. | 2·1 | 0·3 (carbon) |
| Unaccounted .. .. .. | 2·7 | 2·9 |
| Total .. .. .. | 100·0 | 100·0 |

The bulk of the phosphate was ground to 100 per cent. minus 200 mesh, but small samples were prepared of 40-60 mesh and 60-150 mesh. Since all chlorinations were carried out on phosphate that had been ignited to 650°C., the figures cited in Table 1 were used for the calculations given later.

(iii) *Carbon.*

In the preliminary experimental work (see below) activated carbon such as is used for decolorizing purposes was employed.

(iv) *Charcoal.*

Good quality industrial charcoal was ground to 100 per cent. minus 200 mesh, a considerable proportion being finer than this. This was used for all large scale work and a considerable part of the laboratory scale investigation. The same charcoal screened to 1 8-1/16 inch, was also used in the laboratory work.

(v) *Tar.*

The tar used was a light fraction of redistilled vertical retort tar, known in Australia as "No. 1 vertical tar". This material has already been fully described (2).

## 4. Laboratory Scale Chlorinations.

(i) *Laboratory Apparatus.*

The laboratory equipment used has also been fully described elsewhere (2).

(ii) *Chlorination of Precipitated Phosphate.*

Initial experiments were made using the precipitated calcium phosphate and activated carbon described above. These two materials were thoroughly mixed in the proportion 4 : 1 and made into a doughy paste with 2½ per cent. starch solution. This paste was extruded into small pellets about 3/16 in. diameter and ¼ in. long, which were dried

at 120°C. and finally ignited out of contact with the air to about 650°C. to decompose the starch. They were then found not to crumble. Such briquettes gave unsatisfactory results (see, for example, Expt. 1, Table 2), since they were not sufficiently porous for the chlorine to penetrate completely and clogging with calcium chloride occurred. Under the conditions used, viz., with 100 g. of briquettes supported on a bed of silica chips, a temperature of 500°C. and a chlorine rate of 200 cc. per minute it was noted that there was an evolution of water during the first 40 to 60 minutes after which the characteristic odour of phosphorus oxychloride was detected. During this "induction" period presumably the chlorine was adsorbed by the carbon, driving off the adsorbed moisture, and the calcium carbonate contained in the phosphate converted into chloride. The condenser was fitted only after evolution of water ceased, after which the chlorine rate was reduced to 100 cc. per minute. The reaction was fairly rapid at 500°C. and more so at 650°C. Since calcium chloride sinters at 670°C. and fuses at 730–740°C., a temperature of 650°C. was not exceeded. For the analysis of the residue in the furnace the following simple procedure was adopted. The briquettes were leached with boiling water to remove $CaCl_2$ and the insoluble residue, consisting of carbon and unattacked phosphate, was filtered off. The filtrate on evaporation and complete drying, finally at 400–500°C., yielded anhydrous calcium chloride, from which could be calculated the amount of phosphate reacted. The residue was dried at 120°C., weighed, and ignited in a muffle at 700°C. to remove the carbon. Reweighing gave the weight of carbon (by difference) and the weight of unattacked phosphate.

It was found in a typical case (see No. 1, Table 2) that the residue after removal of carbon amounted to 25 g., while the calcium chloride recovered was 54 g., signifying the reaction of 53 g. of "$Ca_3(PO_4)_2$". From these data it appeared that although 55 g. (69 per cent.) of the phosphate had been used up, only 45 per cent. of the theoretically possible oxychloride was condensed. After various sizes of condensers, and the effect of ice-cold cooling liquids, had been tried without any improvement in these figures, it was concluded that the phenomenon was due to the combined water in the phosphate, which, being liberated only during the attack by the chlorine, combined at once with part of the oxychloride. A similar phenomenon was noted when Ocean Island phosphate was chlorinated (see below).

It was thought desirable to investigate two new factors before trying to make briquettes of higher porosity: (*a*) effect of the use of ground charcoal in place of the activated carbon; and (*b*) effect of the use of Ocean Island phosphate in place of the precipitated material.

(iii) *Chlorination of Precipitated Phosphate and Wood Charcoal.*

Briquettes were formed from precipitated calcium phosphate and finely ground "red gum" charcoal as previously described. On chlorination in the furnace no significant difference in behaviour could be noticed. The yield of oxychloride and the chlorine efficiency (No. 9) were the same as before, and it would appear that either (*a*) activated carbon was not necessary, or (*b*) that the charcoal became activated during the "induction" period when it is in contact with chlorine at 500–600°C.

TABLE 2.—SUMMARY OF LABORATORY CHLORINATIONS.

| Expt. No. | Charge, Ignited Weight. | Proportion $Ca_3(PO_4)_2$ : C. by weight. | Type of Briquette. | Phosphate and Mesh. | Carbon and Mesh. | Size of Briquette. | Temperature. | Total Time of Run. | Yield $POCl_3$. | | Chlorine Efficiency. | Residue. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | Weight of Phosphate. | Weight of Carbon. |
| | g. | | | | | | °C. | hours. | g. | percentage on phosphate. | per cent. | g. | g. |
| 1 | 100 | 80 : 20 | Extruded pellet | pptd. —400 ? | Activated, approx. 400 | $\frac{3}{16}$ in. diam., $\frac{1}{4}$ in. long | 550 | 2·7 | 36 | 45·5 | 25 | 25 | .. |
| 2 | ,, | ,, | ,, | ,, | ,, | ,, | 670 | 2 | 35 | 45 ? | 22 | .. | .. |
| 3 | ,, | 73 : 21 | ,, | Ocean Is. —200 | ,, | ,, | 550 | 4 | 11 | 16 | <15 | .. | .. |
| 4 | ,, | ,, | ,, | ,, | ,, | ,, | 670 | 4 | 33 | 42 | 22 | .. | .. |
| 5 | ,, | ,, | ,, | ,, | ,, | ,, | 750 | 2 | 25 | 32 | 25 | .. | .. |
| 6 | ,, | ,, | ,, | Ocean Is. 40—60 | ,, | ,, | 670 | 3·5 | .. | 15 | .. | .. | .. |
| 7 | ,, | ,, | ,, | Ocean Is. 60—150 | ,, | ,, | ,, | 4 | .. | 30 | .. | .. | .. |
| 8 | ,, | ,, | ,, | Ocean Is. —200 | ,, | ,, | ,, | 4 | 33 | 42 | .. | .. | .. |
| 9 | ,, | ,, | ,, | pptd. —400 ? | "Red gum" —200 | ,, | ,, | 4 | 34 | 43 | 24 | .. | .. |
| 10 | 90 | 57 : 29 | Extruded and containing rough charcoal | Ocean Is. —200 | ,, | $\frac{3}{8}$ in. diam., $\frac{1}{2}$ in. long. | ,, | 3·5 | $28\frac{1}{2}$ | 50 | 25 | 22 | 16 |
| 11 | ,, | 63 : 21·6 | Tar-bonded porous | ,, | ,, | $\frac{1}{2}$ in. mesh | ,, | 3·5 | 39 | 63 | 33 | 12 | 13 |
| 12 | ,, | 68 : 16 | ,, | ,, | ,, | ,, | ,, | 3·5 | 30 | 45 | 26 | .. | .. |

(iv) *Chlorination of Ocean Island Phosphate and Wood Charcoal.*

Although this phosphate contained 87·7 per cent. calcium phosphate calculated as $Ca_3(PO_4)_2$, instead of the assumed 100 per cent. of the precipitated powder used previously, the proportions used for briquetting were not altered, and the composition of the air-dried briquettes is given in No. 1, Table 3. After ignition to 650°C., the proportions

TABLE 3.

| No. | Type of Briquette. | Treatment. | Composition of Briquettes. | | | | Theoretical Yield $POCl_3$, Assuming all Phosphorus Converted. |
|---|---|---|---|---|---|---|---|
| | | | Crude Phosphate. | Carbon. | Phosphorus as $Ca_3(PO_4)_2$. | Impurities from Crude Phosphate. | |
| | | | % | % | % | % | g. |
| 1 | Non-porous | Before ignition | 80 | 20 | 70·4 | 9·6 | 69·5 |
| 2 | Non-porous | After ignition to 650 C. | 78·9 | 21·1 | 72·9 | 6 | 72 |
| 3 | Porous .. | After ignition to 650 C. | 76 | 24 | 70 | 6 | 69 |

changed owing to the decomposition of the organic matter in the phosphate and the loss of a certain amount of water, so that the composition changed to that shown in No. 2 of the same table.

On chlorination of these briquettes at 500–600°C. (e.g., No. 3, Table 2) a very small yield resulted in 2 hr. but when treatment was continued for another 2–4 hr., phosphorus oxychloride continued to be evolved slowly. In chlorinations at higher temperatures, a reaction rate comparable to that of the precipitated phosphate at 550°C. was attained only at a temperature of 750°C. (No. 5, Table 2) which is well beyond the practicable limit, namely 670°C. At 670°C. (No. 4, Table 2) the rate was noticeably slower, but the time factor only was involved, since the chlorine efficiency and conversion of the charge were practically the same as in the initial experiments. The time of chlorination was four hours and it was necessary to reduce the chlorine rate accordingly. It may be noted here that moisture was also evolved from the Ocean Island phosphate when chlorine was first passed through the charge, and that the "induction" period was of approximately the same duration.

(v) *Formation of Porous Briquettes.*

Attention was next turned to the formation of briquettes of such porosity that higher yields might be obtained, while at the same time the size of the briquette could be increased by about ten times, a larger unit being considered necessary for pilot plant production. The final aim was to get *all* the phosphate present reacted. Several briquetting methods were tried, among which may be cited: (*a*) the mixing of about

10 per cent. of charcoal pieces screened to $\frac{1}{8}$-1/16 in. mesh with the phosphate and carbon, (*b*) the mixing of a similar quantity of sawdust which was afterwards carbonized, and (*c*) the use of molasses, dextrin, and various other binder solutions instead of starch. Some success was obtained with all these, especially (*a*) which brought the yield up to 50 per cent. on the $Ca_3(PO_4)_2$ content when using briquettes as large as $\frac{3}{8}$-in. diam. and $\frac{1}{2}$-in. long (see No. 10)—a great increase in porosity over the original pellets.

The method finally adopted was one that has been described previously (2). On chlorination in the laboratory furnace at 670°C. (see No. 11) such briquettes appeared far more reactive than any of the other types. It will be seen that the chlorine efficiency rose to 33 per cent., and 63-65 per cent. of the phosphatic content was utilized to give *recovered* phosphorus oxychloride; 18 per cent. was unattacked, being recovered in the residue. The reaction time was only about 50 per cent. greater than that for the precipitated phosphate and therefore was not unduly long. The composition of the charge is given in No. 3, Table 3.

The only other comment that need be made concerns the high proportion of carbon in these briquettes due to the additional amount derived from the carbonized tar. It was approx. 1:3 instead of 1:4, and in No. 11, Table 2, of the 21·6 g. of carbon present in the charge before chlorination, 13 g. were recovered, together with 12 g. of phosphate. Hence it appeared that it would be possible to cut down the proportion to, say, 0·7 : 4, and still have ample. However, it was found that this excess carbon was beneficial in the reaction, and brought about considerably faster production of phosphorus oxychloride than was obtained when the proportion of carbon to phosphate was near to 0·7 : 4, see No. 12, Table 2. This was probably due to the excess carbon ensuring a uniform concentration of reducing gas, together with the added porosity given to the briquettes by the carbon particles.

The above data concerning the proportion of carbon in the residue provided the evidence previously mentioned that pointed to the formation of a certain amount of carbon dioxide, as well as carbon monoxide, during the reaction. Thus in No. 11, 51 g. of phosphate was consumed together with 8·6 g. of carbon. If carbon monoxide only had been formed 12·5 g. of carbon would have reacted, while, if only carbon dioxide had been formed, this figure would have been 6·25 g. The intermediate value found experimentally pointed to a mixture of the two gases being produced.

(vi) *The Effect of Particle Size of Phosphate on the Reaction Rate.*

The laboratory-scale experimental work was concluded with an investigation into the effect of particle size of the phosphate on the yield. For this purpose starch-bonded pellets were formed with Ocean Is. phosphate and carbon in the proportions 4 : 1, the phosphate being screened to (*a*) 40-60, (*b*) 60-150, and (*c*) —200 mesh. The results, which are tabulated as Nos. 6, 7, and 8 (Table 2), show that particle size was critical; at least —200 mesh phosphate was essential for a reasonably fast rate of reaction. This probably indicates why the precipitated phosphate was so much superior in this respect, for it was all —350 mesh.

*(vii) Purification of Phosphorus Oxychloride.*

Phosphorus oxychloride obtained from the chorination of precipitated calcium phosphate was pale-yellow in colour, almost entirely caused by dissolved chlorine. On boiling for a short time this chlorine was practically eliminated, so that on distillation an almost water-white product resulted. The last traces of chlorine were removed by carrying out the distillation over copper. Oxychloride from the chlorination of Ocean Is. phosphate in the form of tar-bonded briquettes was orange to dark-red, owing to traces of ferric chloride and chlorine, as well as dissolved chlorine. A water-white product was obtained by refluxing over copper foil before distillation, but if the crude furnace product was very dark, it was preferable to distil before treating with copper.

*(viii) Summary of Laboratory Scale Chlorinations.*

The experimental laboratory work may be briefly summarized as follows:—

(*a*) Up to 80-90 per cent. of the phosphatic content of the charge was converted by chlorine, but not more than 65 per cent. was obtained as phosphorus oxychloride. This low yield appeared to be due to the combined water in the phosphate which was liberated during chlorination and destroyed part of the oxychloride. This loss was approximately one-quarter in the Ocean Is. phosphate and one-third in the precipitated phosphate.

(*b*) The temperature at which the Ocean Is. phosphate was chlorinated was maintained close to 670°C., without exceeding this value. Evenness of heating was therefore essential, and the necessary steps were taken to eliminate any temperature gradient along the furnace. Slight sintering occurred at this temperature but it was never enough to prevent easy removal of the residue.

(*c*) The chlorine efficiency did not exceed 33 per cent. (cf. 50 per cent. from theoretical equation). Part of this loss was explained, in the case of Ocean Is. phosphate, by the fact that this material contained 3·6 per cent. of calcium carbonate and 0·8 per cent. of ferric oxide which also reacted with chlorine. The remainder was due to the failure (described above) to recover one-quarter of the phosphorus oxychloride.

## 5. Pilot Plant Chlorinations.

The pilot plant equipment and mode of operation have also been fully described elsewhere (2). Although no changes were needed for the production of phosphorus oxychloride, it was found that the temperature varied along the furnace to such an extent that only the central 12 inches could be maintained within the usual temperature range of 650-670°C. Hence the capacity of the furnace was somewhat reduced by the necessity to build up a thicker bed of silica chips at the bottom and to limit the size of charge to about 30 lb. so that it lay within the zone of uniform temperature. The briquettes were ignited at 700°C. in the furnace itself in a slow stream of nitrogen, and after all volatiles

had been driven off, the temperature was allowed to fall to 670°C. when chlorine was passed at 8 lb. per hour. After an "induction" period of approximately 1·3 hours, phosphorus oxychloride was evolved. Water vapour, which was by-passed from the condenser and allowed to escape to the air, was evolved earlier. The condenser was then connected, the chlorine rate lowered to 4 lb./hr., and the oxychloride soon commenced to flow into the receiver. After a further period of 4-5 hours the rate of formation of the phosphorus oxychloride fell noticeably, and the chlorine rate was lowered to 2½ lb. per hour for one hour. Finally, nitrogen was blown through the furnace for some time until the last traces of product had been swept over. The data for a typical chlorination are given in Table 4.

Table 4.—Data from Typical Pilot Plant Chlorination.

| | |
|---|---|
| Experiment No. | 4 |
| Briquette Type and Size | Tar-bonded porous, –½-in. mesh |
| Temperature Range | 650-670°C. |
| Charge Weight | 30 lb. |
| $Ca_3(PO_4)_2$ Content | 22 lb. |
| $POCl_3$ Theoretical | 19·4 lb. |
| $POCl_3$ Obtained | 12·4 lb. |
| Percentage $POCl_3$ on Phosphate | 64 |
| $Cl_2$ Used | 26·5 lb. |
| $Cl_2$ Efficiency | 32 per cent. |
| $Ca_3(PO_4)_2$ in Residue | 2·2 lb. |
| Efficiency on $Ca_3(PO_4)_2$ | 90 per cent. |

It will be noticed that the efficiency figures obtained in the laboratory experiments were almost duplicated in the larger scale production.

## 6. Estimation of Costs Based on Raw Materials.

The following costs of raw materials were taken for this estimate:—

| | |
|---|---|
| Chlorine (in lots greater than 1 ton) | £35 per ton. |
| Phosphate (Ocean Is.) | £8 per ton. |
| Grinding phosphate to 200 mesh | £10 per ton. |
| Black coal (ordinary bituminous) | £2 3s. per ton. |
| Grinding coal to 150 mesh | £1 per ton. |
| Tar | £8 |

Based on these alone, and excluding all other costs, it was estimated that the materials required for its production would cost 1s. 3d. per lb. of phosphorus oxychloride produced. This price may be compared to the selling price of oxychloride in England at the same period which was quoted as 11d. per pound, i.e., 1s. 2d. Australian currency.

## 7. References.

(1) U.S. Patent 1,462,732 (1923)
Fr. Patent 769,702 (1924)
Brit. Patent 336,065 (1930)
Brit. Patent 337,123 (1930)
Brit. Patent 416,084 (1934)

(2) McTaggart, F. K.—*J. Coun. Sci. Ind. Res.* (Aust.) **18**: 5 (1945).

# A Qualitative Test for Cement-aggregate Reaction.

*By A. R. Alderman, Ph.D., D.Sc.,* A. J. Gaskin, M.Sc.,* and H. E. Vivian, B.Sc.Agr.**

*Summary.*

Expansive reaction between its cement and aggregate components may lead to deterioration of concrete. A simple method for the recognition of dangerous cement-aggregate combinations is described.

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The procedure described in this paper was developed in an attempt to devise a test which would give a direct indication of expansive reaction between cement and aggregate in concrete. It was desired that such a test should not only give direct evidence of expansion in a cement-aggregate combination but should be obtainable by simple apparatus and procedure, and should also, if possible, be rapid in action.

The test is based upon the distortion produced in a body undergoing differential expansion. The test pieces which we have found to be of convenient size consist of thin slabs of cement mortar measuring 4 in. by 2 in. by $\frac{1}{4}$ in., in which a material known to be non-reactive is used as aggregate on one side and the aggregate to be tested is used on the other (Fig. 1). The slab thus consists of two layers each measuring 4in. by 2 in. by $\frac{1}{8}$ in. made from the same cement but different aggregates.

Fig. 1.—Bi-aggregate slab showing the two layers of mortar. No distortion or damp spots are shown by this specimen.

The mould for the fabrication of the bi-aggregate slabs consists of a flat base-plate on to which two flat L-shaped side-pieces, $\frac{1}{8}$ in. thick and alternately tongued, fit together to give an open space of 4 in. by 2 in. by $\frac{1}{8}$ in. The side-pieces are kept in position by vertical pins

* An officer of the Division of Industrial Chemistry.

which are screwed into the base-plate and which fit into holes in the tongued ends of the side-pieces. The mould at this stage is ready for the fabrication of a layer of mortar measuring 4 in. by 2 in. by $\frac{1}{8}$ in. (Fig. 2). When this first layer has been made, two further identical side-pieces are fitted on top of the first ones, and a second layer of mortar of identical dimensions can be placed directly on the first layer. The final slab then measures 4 in. by 2 in. by $\frac{1}{4}$ in.

Fig. 2.—Mould for fabrication of slabs. Side-pieces for the lower layer are in position. The upper pair of side-pieces is in the foreground.

A sufficient amount of mortar for the fabrication of each layer of the slab can be made by mixing 15 g. of cement, 30 g. of aggregate, and $7\frac{1}{2}$ ml. of water. These proportions have been used for all mortars prepared for the present investigation. Leighton Buzzard sand† (18–25 B.S.S. mesh) has been used throughout as the non-reactive aggregate. Test aggregates are generally crushed and sieved to 18–25 or 18–52 B.S.S. mesh. Trials made using a non-reactive aggregate, crushed to different gradings, on opposite sides of the slab showed that no perceptible distortion was produced by varying grain-size. Further trials also showed that it is immaterial which mortar is placed in the lower part of the mould, similar results being obtained from slabs of identical composition but moulded in the reverse order.

Standard procedure is followed in mixing and moulding the mortar for the thin slabs. After the lower layer has been moulded the top is levelled by means of the trowel or palette-knife, the top of the lower side-pieces is wiped clean, and the upper side-pieces are fitted into position. The mixing and the moulding of the upper layer of mortar is commenced immediately, care being taken to avoid disturbing the lower layer. The final surface is smoothed to give a slab of as uniform thickness as possible.

† A clear rounded quartz sand from Leighton Buzzard, England, used in Australia as well as in Britain as a standard in cement testing.

After curing in the moist cabinet for 24 hours at 70°F. the mould is taken to pieces and the slab removed. If the mould has been greased before use and a piece of tissue paper placed on top of the base-plate the cured slab can be removed easily. The slabs are stored in moist air in sealed containers, these conditions being similar to those which Stanton (1940)* has found to be most suitable for the promotion of cement-aggregate reaction. Slabs have been stored under these conditions at two temperatures—at room temperature, which in Melbourne would average about 58–59°F., and at 110°F.

In establishing the usefulness of bi-aggregate slabs for testing purposes, a mixture of 10 per cent. of siliceous magnesian limestone from California and 90 per cent. Leighton Buzzard sand was used as the reactive aggregate. Several Australia aggregates containing opaline silica which behave similarly to the Californian rock have since been found.

## Storage at Room Temperature.

Invariably the first indication of a reaction is given by the development of damp-looking spots on the surface of the mortar (Fig. 3). These spots are believed to be due to the formation of silicate gel around

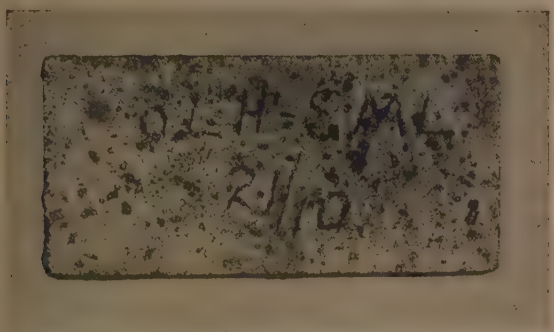

Fig. 3.—Surface of bi-aggregate slab showing damp spots produced by reactive aggregate. The other side of this slab, made with non-reactive sand, shows no spots.

grains of reactive aggregate (Meissner, 1941, p. 554).† The number, size, and rapidity of development of the spots gives a rough indication of the expansivity of the mortar. Although all slabs which contained reactive Californian aggregate eventually developed spots, the period of development varied from six days for highly expansive mortars to 80 days for mortars which showed no visible expansion. Expansion is shown by bending of the slab and is best observed by placing a straight-edge along the long axis of the slab and on the side containing non-reactive aggregate. Slabs matured at room temperature showed bending

* Stanton, T. E. (1940).—*Proc. Amer. Soc. Civil Eng.* **66**: 1781.

† Meissner, H. S. (1941).—*Proc. Amer. Concrete Inst.* **37**: 549.

TABLE 1.—BEHAVIOUR OF BI-AGGREGATE SLABS AT ROOM TEMPERATURE.

*Test Aggregate* = 10 *per cent. Californian siliceous magnesian limestone* + 90 *per cent. Leighton Buzzard sand.*
*Inert Aggregate* = *Leighton Buzzard sand.*

| Cement. | Spots. | | Bending. | | $Na_2O$ in cement. | $K_2O$ in cement. | $Na_2O + K_2O$ (as $Na_2O$). | Expansion Measured. | |
|---|---|---|---|---|---|---|---|---|---|
| | Appeared. | Prominent. | Appeared. | Prominent. | | | | Three Months. | Twelve Months. |
| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) | (9) |
| | Days. | Days. | Days. | Days. | % | % | % | % | % |
| A .. | 81 | .. | .. | .. | ·00 | ·18 | ·12 | ·00 | ·01 |
| B .. | 33 | .. | .. | .. | ·00 | ·38 | ·25 | ·01 | ·07 |
| C .. | 55 | .. | .. | .. | ·00 | ·39 | ·26 | ·01 | ·05 |
| D .. | 17 | .. | .. | .. | ·00 | ·45 | ·30 | ·00 | ·02 |
| E .. | 39 | .. | .. | .. | ·04 | ·53 | ·39 | ·01 | ·09 |
| F .. | 24 | 30 | .. | .. | ·41 | ·00 | ·41 | ·08 | ·33 |
| G .. | 8 | ? | 39 | 64 | ·00 | ·62 | ·41 | ·22 | ·68 |
| H .. | 11 | 20 | 40 | .. | ·00 | ·70 | ·46 | ·16 | ·37 |
| I .. | 9 | 9 | 27 | 40 | ·05 | ·72 | ·52 | ·37 | ·53 |
| J .. | 17 | 31 | 52 | .. | ·17 | ·60 | ·57 | ·33 | ·42 |
| K .. | 11 | 16 | 51 | 76 | ·18 | ·63 | ·60 | ·45 | ·52 |
| L .. | 21 | 26 | 96 | .. | ·59 | ·09 | ·65 | ·20 | ·51 |
| M .. | 6 | 13 | 38 | 46 | ·56 | ·45 | ·86 | ·46 | ·52 |
| N .. | 6 | 20 | 34 | .. | ·51 | ·88 | 1·09 | ·09* | ·11 |

* A mortar bar, made subsequently to check these figures, showed an expansion of 0·44 per cent. at three months. The reason for the small expansion shown in the above table is unknown.

after about 30 days in the case of highly expansive mortars. Results of our observations on slabs made with reactive Californian aggregate and 14 Australian cements are given in Table 1. The cements are arranged in order of increasing alkali content ($Na_2O + K_2O$ expressed as $Na_2O$). Column 1 shows the period after fabrication when spots were first observed on the reactive mortars. Column 2 indicates when, owing to increasing size and numbers, the spots became very prominent.

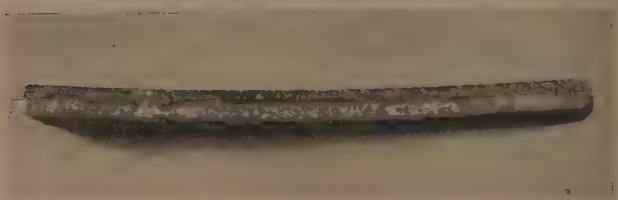

FIG. 4.—Bending of bi-aggregate slab due to expansion of one layer of mortar.

Although such an observation has little claim to exactness, the figures give an inverse indication of the degree of spotting achieved by the various mortars. Column 3 indicates when bending of the slab was first observed. This earliest indication is only observed by placing a straight-edge along the surface of the slab. Later, as bending increases, the curvature of the slab is immediately apparent (Fig. 4). The period of development of such prominent bending, although inexact, is nevertheless instructive and is given in column 4. $Na_2O$ and $K_2O$ contents of the cements are given in columns 5 and 6. Column 7 shows the

$Na_2O$ equivalent of $Na_2O + K_2O$. Columns 8 and 9 are included in order to compare the behaviour of bi-aggregate slabs with the measured changes in length of mortars made from the same series of cements and reactive aggregate. The mortars were made into 10-in.* bars, stored at room temperature in moist air in sealed containers and measured at monthly intervals. The mean percentage linear expansions of duplicate bars at three and twelve months are given.

The details given in Table 1 point to the following conclusions:—

(1) According to their behaviour as mortars with the Californian reactive aggregate, the cements are divisible into two groups; cements A to E, all of which have a low alkali content (less than 0·40 per cent. $Na_2O + K_2O$ as $Na_2O$), and cements F to N, all of which have a higher alkali content.

(2) Bi-aggregate slabs show little evidence of further reaction after three or four months. On the other hand, the more massive 10 in. by 1 in. by 1 in. test bars continue to expand for a much longer period. Behaviour of bi-aggregate slabs shows a reasonably close correlation with measured expansions.

(3) Bi-aggregate slabs consisting of mortar which has an expansion of 0·08 per cent. or less in three months have not bent. Mortars which have an expansion higher than 0·08 per cent. have all shown bending.

(4) There is a close correlation between the development of spots, bending, alkali content, and the measured expansion of mortars.

(5) The former points may be summarized by saying that observation of the development of spots and bending in bi-aggregate slabs gives a sufficiently reliable indication of expansive reaction in mortars.

### Storage at 110 Degrees F.

The same cements and aggregates were made into mortar slabs and the containers stored at 110°F. Table 2 shows that the appearance of spots and bending was greatly accelerated.

From these observations we can draw the following conclusions:—

(1) The division of the cements into a low-alkali group (cements A to E) and the high-alkali group (cements F to N) is again clearly marked.

(2) Slabs made from cements of the high-alkali group developed spots in two or three days; most of them bent within a week.

(3) Slabs made from cements of the low-alkali group took six or more days to develop spots. Most of them did not bend, D being the exception.

(4) Observations made on slabs stored at 110°F. confirm those made on slabs stored at room temperature. Reaction is, however, much more rapid at 110°.

* Overall dimensions of these bars are 11¼ in. by 1 in. by 1 in. The effective length of the bars is, however, 10 in. which is the distance between the embedded gauge points.

TABLE 2.—BEHAVIOUR OF BI-AGGREGATE SLABS AT 110°F.

*Test Aggregate* = 10 *per cent. Californian siliceous magnesian limestone* + 90 *per cent. Leighton Buzzard sand.*

*Inert Aggregate* = *Leighton Buzzard sand.*

| Cement. | Spots. | | Bending. | |
|---|---|---|---|---|
| | Appeared. (1) | Prominent. (2) | Appeared. (3) | Prominent. (4) |
| | Days. | Days. | Days. | Days. |
| A .. .. | 11 | .. | .. | .. |
| B .. .. | 6 | 7 | .. | .. |
| C .. .. | 9 | .. | .. | .. |
| D .. .. | 7 | .. | 40 | .. |
| E .. .. | 8 | .. | .. | .. |
| F .. .. | 3 | 7 | 23 | 37 |
| G .. .. | 2 | 7 | 19 | 28 |
| H .. .. | 2 | 5 | 6 | 21 |
| I .. .. | 2 | 8 | 6 | 7 |
| J .. .. | 3 | 4 | 7 | 10 |
| K .. .. | 3 | 4 | 7 | 8 |
| L .. .. | 3 | 7 | 7 | 16 |
| M .. .. | 2 | 2 | 3 | 4 |
| N .. .. | 2 | 2 | 3 | 3 |

## Examination of Australian Aggregates.

The method has been applied to many Australian aggregates. In the great majority of cases results have been negative, suggesting that the aggregates are non-reactive, a fact confirmed by subsequent measurement of 10-in. bars over a period of one to two years. The following results are typical but include in Tables 3 and 4 tests made on two

TABLE 3.—AGGREGATE S14, OPALINE QUARTZITE ("GIBBER"), OODNADATTA, SOUTH AUSTRALIA.

*Storage at 110°F.*

| Cement. | Spots Appeared. (1) | Bending. | | $Na_2O$ in Cement. (4) | $K_2O$ in Cement. (5) | $Na_2O + K_2O$ (as $Na_2O$). (6) | Expansion at Room Temp. | |
|---|---|---|---|---|---|---|---|---|
| | | Appeared. (2) | Prominent. (3) | | | | Three Months. (7) | Twelve Months. (8) |
| | Days. | Days. | Days. | % | % | % | % | % |
| A .. | 47 | .. | .. | ·00 | ·18 | ·12 | ·00 | ·02 |
| J .. | 13 | 41 | .. | ·17 | ·60 | ·57 | ·02 | ·05 |
| N .. | 18 | 46 | 85 | ·51 | ·88 | 1·09 | ·03 | ·07 |

aggregates which contain opal and show significant expansions when used with high-alkali cements. When these slabs are stored at 110°F. the early development of spots is very difficult to observe, owing to the relative roughness and dampness of the surface of the mortar: the figures given in column 1 of the tables are therefore only approximations. Leighton Buzzard sand was used as the inert aggregate in all cases. Slabs were stored at 110°F. and, for purposes of comparison, the measured expansions of corresponding 10-in. mortar bars stored at room temperatures are given.

TABLE 4.—AGGREGATE S15, OPALINE QUARTZITE, COOBER PEDY, SOUTH AUSTRALIA.

*Storage at 110°F.*

| Cement. | Spots Appeared. | Bending. | | $Na_2O$ in Cement. | $K_2O$ in Cement. | $Na_2O + K_2O$ (as $Na_2O$). | Expansion at Room Temp. | |
|---|---|---|---|---|---|---|---|---|
| | | Appeared. | Prominent. | | | | Three Months. | Twelve Months. |
| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) |
| | Days. | Days. | Days. | % | % | % | % | % |
| A .. | 50 | .. | .. | ·00 | ·18 | ·12 | ·01 | ·02 |
| J .. | 14 | 14 | 23 | ·17 | ·60 | ·57 | ·02 | ·07 |
| N .. | 18 | 18 | 46 | ·51 | ·88 | 1·09 | ·15 | ·26 |

Tables 5 and 6 give results typical of nearly all the Australian aggregates which were examined. Tables 3 and 4 suggest that the two opaline aggregates would give trouble if used with high-alkali cement

TABLE 5.—AGGREGATE T5, DOLERITE, BUTLER'S GORGE, TASMANIA.

*Storage at 110°F.*

| Cement. | Spots Appeared. | Bending. | | $Na_2O$ in Cement. | $K_2O$ in Cement. | $Na_2O + K_2O$ (as $Na_2O$). | Expansion at Room Temp. | |
|---|---|---|---|---|---|---|---|---|
| | | Appeared. | Prominent. | | | | Three Months. | Twelve Months. |
| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) |
| | Days. | Days. | Days. | % | % | % | % | % |
| A .. | .. | .. | .. | ·00 | ·18 | ·12 | ·00 | ·01 |
| J .. | .. | .. | .. | ·17 | ·60 | ·57 | n.d. | n.d. |
| N .. | .. | .. | .. | ·51 | ·88 | 1·09 | ·00 | ·02 |

TABLE 6.—AGGREGATE T7, BLYTH SAND, N.W. TASMANIA.

*Storage at 110°F.*

| Cement. | Spots Appeared. | Bending. | | $Na_2O$ in Cement. | $K_2O$ in Cement. | $Na_2O + K_2O$ (as $Na_2O$). | Expansion at Room Temp. | |
|---|---|---|---|---|---|---|---|---|
| | | Appeared. | Prominent. | | | | Three Months. | Twelve Months. |
| | (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) |
| | Days. | Days. | Days. | % | % | % | % | % |
| A .. | .. | .. | .. | ·00 | ·18 | ·12 | ·00 | ·02 |
| J .. | .. | .. | .. | ·17 | ·60 | ·57 | n.d. | n.d. |
| N .. | .. | .. | .. | ·51 | ·88 | 1·09 | ·00 | ·02 |

These tables also show that the observation of bi-aggregate slabs stored at 110°F. does not always give results which are in direct relationship with those obtained by measurement of mortar bars stored at room temperature, as in the test originated by Stanton.

In attempting to evaluate the results obtained from all our observations on bi-aggregate slabs, we can state the following two facts:—

(1) Bi-aggregate slabs have not failed to indicate expansive reaction in mortars which at atmospheric temperatures have developed significant expansions in twelve months.

(2) In no instances have bi-aggregate slabs given indications of expansive reaction which were not fully substantiated by other observations.

Although the number of aggregates which are known to be reactive and which we have been able to examine is small, the bi-aggregate slab test has, within the limits of this investigation, given reliable indications of expansive reaction in mortars. Instances are known, however, in other parts of the world where expansive deterioration has not become evident till many years after the concrete has set. Little is known about this long-term type of reaction and, as aggregates associated with it have not been available for investigation, it is not claimed that bi-aggregate slabs will assist in its diagnosis. The method has, however, shown its reliability when applied to known aggregates, the apparatus required is simple, and storage at elevated temperature (e.g., 110°F.) has given results in a few days or weeks.

## Acknowledgments.

We are indebted to Mr. T. E. Stanton and the California Division of Highways for samples of reactive aggregates from California; to officers of the South Australian Department of Mines, the Commonwealth Railways, the Tasmanian Hydroelectric Commission, and to Mr. L. R. Davies Graham for samples of Australian aggregates.

We are also grateful for the financial assistance and co-operation of the Australian Cement Manufacturers' Association.

# NOTES.

## The Lord Rutherford Memorial Research Fellowship.

The Canterbury University College, Christchurch, New Zealand, is calling for applications for the above Fellowship, founded in 1941 to commemorate the late Baron Rutherford of Nelson, a former graduate of the College. An award is to be made every second year.

The Fellowship is open to graduates of the University of New Zealand or to those of any other university within the British Empire who have resided in New Zealand for a period of not less than three years, and is awarded for outstanding merit and promise in the subjects Physics, Chemistry, and Mathematics, or any one of these subjects.

The Fellowship is awarded for the purpose of giving the Fellow an opportunity for further study or of carrying out research work at some institution approved by the Selection Committee.

The tenure of the Fellowship is normally two years but in special cases may be extended for a third year. The annual value is approximately £400 (not less than £375) New Zealand currency.

The Fellow must devote himself wholly to the objects of the Fellowship and is debarred from holding any position of emolument except by permission of the Selection Committee.

Applications must reach the Canterbury University College, Christchurch, New Zealand, not later than January 31, 1946.

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## Reviews.

"Collected Papers on Metallurgical Analysis by the Spectrograph," edited by D. M. Smith.

(Published by the British Non-Ferrous Metals Research Association, Euston Street, London, N.W.1, 1945. Pp. X + 162, 58 figs. Price 21s. English.)

For some years past the B.N.F.M.R.A. Sub-Committee on Metallurgical Applications of the Spectrograph has operated through various panels covering different aspects of the work. Apart from guiding the Association's own spectrographic researches the individual members of the panels have also provided results obtained in their own laboratories. In this way a considerable number of reports have been distributed to the B.N.F.M.R.A. membership, some emanating from the Association's own research staff and some from laboratories of member companies.

The present volume contains a selection of thirteen papers based on these various reports: two on the processing and calibration of the photographic plate; four on analysis of aluminium and aluminium alloys; three on lead and lead alloys; one on zinc alloys; two on copper alloys; and one on platinum. The co-operative nature of this work is shown by the fact that of these thirteen papers, six are from members of the Association's staff, three from member companies, one from a Government Department, and three are reports from panels of the B.N.F.M.R.A. Sub-Committee.

The book is not a systematic treatise on the spectrographic analysis of metals and alloys, but many of the papers make practical recommendations on various aspects of technique. The papers cover a wide range and the book will be of great interest to all engaged in the spectrographic analysis of non-ferrous metals.

"Co-operation in Forestry," by I. Kissin, M.A., D.Phil.

(Technical Communication No. 2 of the Imperial Forestry Bureau, 1944. Pp. 72. Price 4s. Copies obtainable from Imperial Agricultural Bureaux, Central Sales Branch, Penglais, Aberystwyth, Wales.)

Co-operative organization is important as a means for the advancement of private forestry. This Communication makes a survey of co-operative societies in forestry on the basis of a comparatively wide range of evidence obtained from the literature and by direct enquiry.

Chapter I. is a general discussion of the nature of co-operation and of the main categories of co-operative effort in forestry, and of the activities, organization, and finance of societies of forest owners.

Chapters II.-V. contain a detailed study of the existing British co-operative forestry societies and a fairly detailed account of the societies of forest small-holders in Denmark, the forest management societies of Finland, and certain farm-woodland co-operatives in the U.S.A. In each case consideration is given to the forestry background against which the societies have developed, and to their history, organization, finance, membership, and activities.

A summary of available information on other societies of forest owners, and on certain agricultural co-operative societies that market forest produce, is given in Chapter VI. This covers various organizations in European countries, notably Sweden, Norway, and Finland, and in the U.S.A., Canada, and Japan.

Co-operation among forest workers is discussed in Chapter VII. The important groups of organizations in Roumania, Bulgaria, and the U.S.S.R. receive special attention; other societies described include several in Quebec and one of special interest in the Gold Coast.

In the Appendix a review is given of certain forestry organizations that are termed "quasi-co-operative." They include the German "Waldgenossenschaften," some of which date back to the Middle Ages, the "composesorate" in Roumania, and "compulsory forestry co-operatives" in Greece and Japan.

The Communication concludes with a bibliography of over 140 references cited in the text, and with summaries in Portuguese and Spanish.

"Forestry Credit," by I. Kissin, M.A., D.Phil.

(Technical Communication No. 3 of the Imperial Forestry Bureau, 1944. Pp. 27. Price 2s. 6d.)

Lack of financial resources often hinders the practice of efficient forest management. The provision of forestry credit thus deserves consideration as a means for the advancement of private forestry.

The present Communication, based on a comparatively wide range of evidence, is a study of the special features of, and some of the experience gained with, forestry credit.

Chapers I. to IV. are general. The functions of forestry credit and the special problems associated with the mortgage of forests are discussed, and a classification is made of the various types of credit

employed in forestry production. Some general trends in the provision of forestry credit are outlined with reference to the type of credit institutions that have advanced forestry loans, the restrictions often imposed on these loans, and the provision and use of subsidized forestry credit and of afforestation credit.

Chapters V. and VI deal with the position in a few countries in which developments of interest have taken place, including Germany, Norway, Denmark, and the U.S.A. The very significant official proposals made in the U.S.A. for the improvement of forestry credit facilities, aiming *inter alia* at establishing a Forest Credit Bank that would embody a forest insurance organization, are discussed in some detail.

Chapter VII. includes notes on credit in British forestry and on forest mortgage credit in Canada, South Africa, and Australia.

The Communication concludes with summaries in Portuguese and Spanish and a bibliography of sixty references cited in the text.

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## Recent Publications of the Council.

Since the last issue of this *Journal*, the following publication of the Council has been issued:—

*Bulletin No.* 185.—"Studies on the Mitchell Grass Pasture in South-Western Queensland. 2. The Effect of Grazing on the Mitchell Grass Pasture," by R. Roe, B.Sc.(Agric.), and G. H. Allen, Dip. Agric. (Lawes).

This Bulletin is a progress report of grazing trials on a natural Mitchell grass pasture in south-western Queensland. Three rates of stocking were compared: a light rate of one sheep to 7½ acres, a heavy rate of one sheep to 2½ acres, and a medium rate of one sheep to 5 acres, which experienced graziers consider to be the approximate carrying capacity of the particular type of Mitchell grass pasture being used. Each rate of stocking was tested under continuous grazing and under a rotational system, whereby a paddock was grazed at double rate for six months and then spelled for six months.

The trial has been in progress for three years, but it must be continued for some years before definite conclusions can be drawn. Tentative results show that continuous grazing at the medium rate was the best treatment tested. Under it there was no evidence of pasture deterioration, and sheep liveweights and wool production were maintained almost as well as under light-grazing at one sheep to 7½ acres.

As part of a system of general management, the regular practice of heavily grazing Mitchell grass pasture for a lengthy period over the summer months, particularly during summers of light rainfall, should be avoided; it leads to elimination of the Mitchell grass from the pasture.

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## Forthcoming Publications of the Council.

At the present time, the following future publications of the Council are in the press:—

*Bulletin No.* 186.—"The General Ecological Characteristics of the Outbreak Areas and Outbreak Years of the Australian Plague Locust (*Chortoicetes terminifera* Walk.)," by K. H. L. Key, M.Sc., Ph.D.

*Bulletin No.* 187.—"Alcohol: Its Place in Synthetic Organic Chemical Industry," by H. H. Hatt, B.Sc., Ph.D.

*Bulletin No.* 188.—"A Soil, Land-Use, and Erosion Survey of County Victoria, South Australia, including the Hundreds of Belalie, Whyte, Reynolds, and Anne and part of the Hundreds of Caltowie, Yangya, and Bundaleer," by C. G. Stephens, M.Sc., R. I. Herriot, B.Ag.Sc., R. G. Downes, M.Agr.Sc., T. Langford-Smith, M.Sc., and A. M. Acock, B.A., D.Phil.

*Bulletin No.* 189.—"Soils of the Berriquin Irrigation District of N.S.W.," by Robert Smith, B.Sc. (Agric.).

*Bulletin No.* 190.—"Foundry Sand Resources of South Australia," by H. S. Cornelius and H. A. Stephens, B.Sc.

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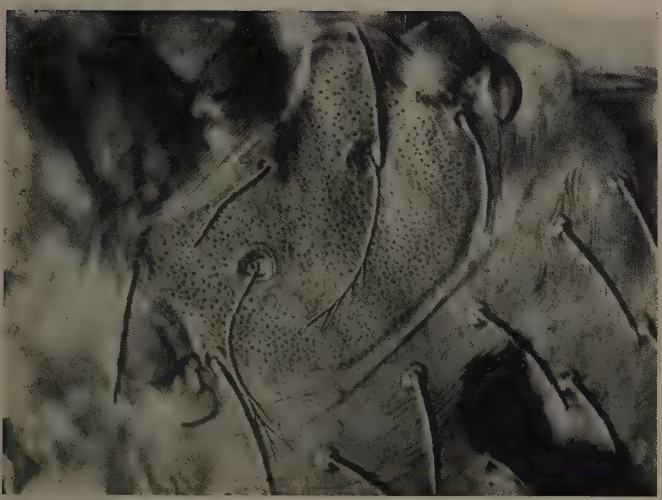
**PLATE 1.**

Microphotography as an Aid to the Identification of Trombiculine Larvae. (See page 298.)



*Trombicula sarcina.* Wom. 1944. Central Highlands of Queensland. Mite from soil Mag. 100 ×. Dorsal Scutum Mag. 610 ×.

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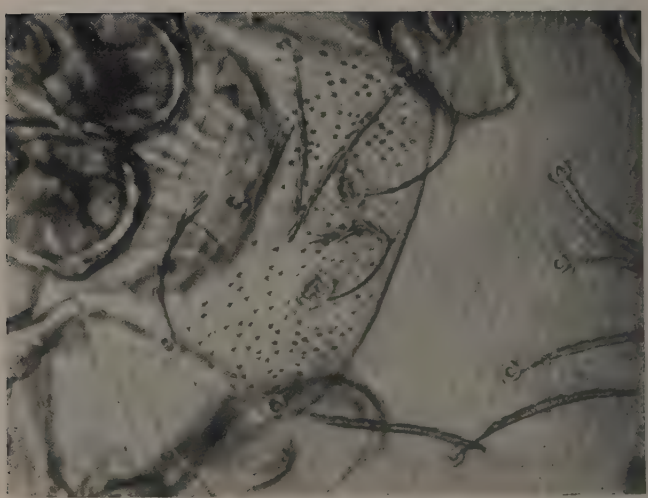
**PLATE 2.**

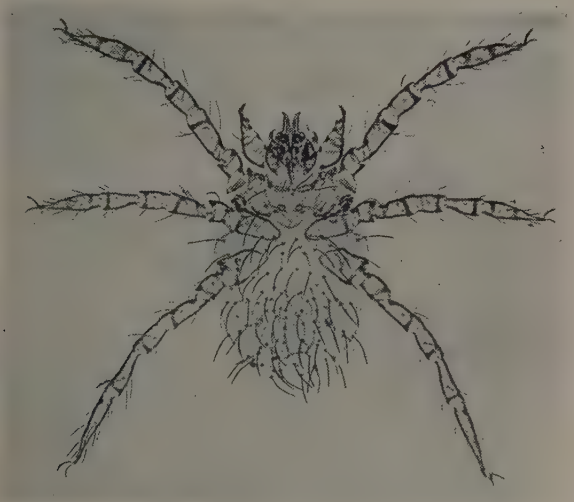
Microphotography as an Aid to the Identification of Trombiculine Larvae. (See page 298.)



New species of *Trombicula*, con mon on native birds Central Queensland. Mite partially engorged Mag. 100 ×. Scutum Mag. 610 ×.

**PLATE 3.**

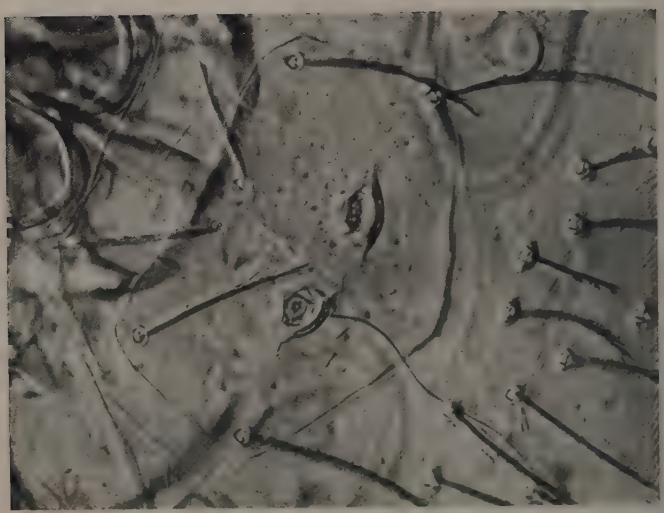
Microphotography as an Aid to the Identification of Trombiculine Larvae. (See page 298.)



*Leeuwenhoekia adelaideae.* Wom. 1944. Central Highlands of Queensland.
Mite from soil Mag. 100 ×. Scutum Mag. 610 ×

## PLATE 4.

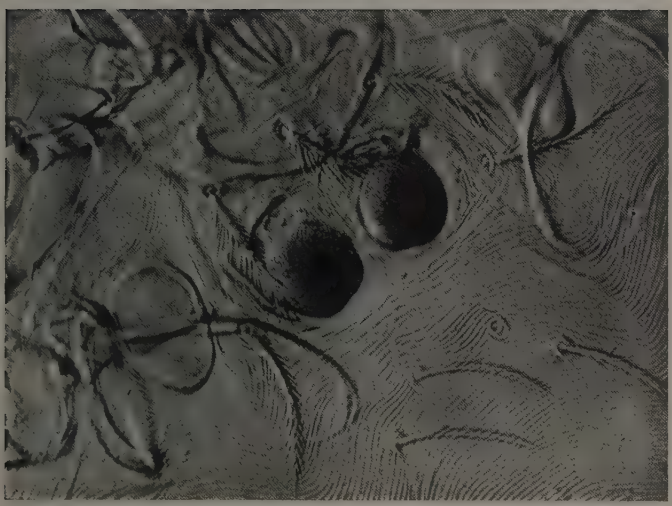
Microphotography as an Aid to the Identification of Trombiculine Larvae. (See page 298.)



*Leeuwenhoekia australiensis.* Hirst 1925. From cat at Chatswood, Sydney. Mite had commenced to engorge. Slightly distorted during removal. Mag. 100 ×. Scutum 610 ×. Note the clearly defined anterior border both here and in Plate 3, also the striking difference in size of the anterior process in the two species.

**PLATE 5.**

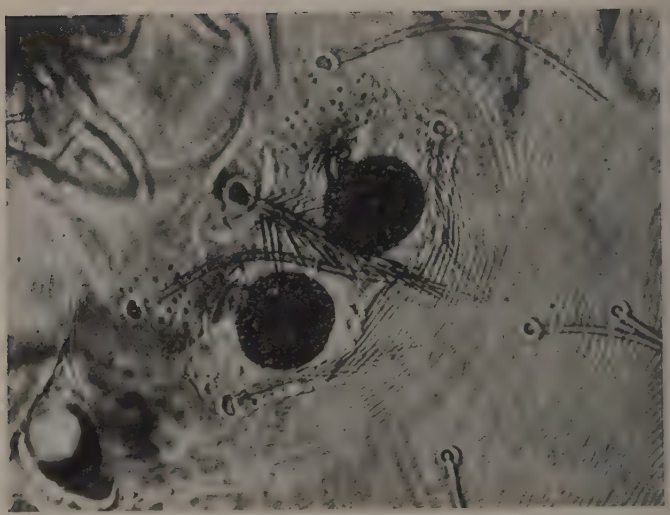
Microphotography as an Aid to the Identification of Trombiculine Larvae. (See page 298.)



New Species of *Paraschongastia* common on native birds Central Queensland. Mite semi-engorged Mag. 100 ×. Scutum Mag. 610 ×.

## PLATE 6.

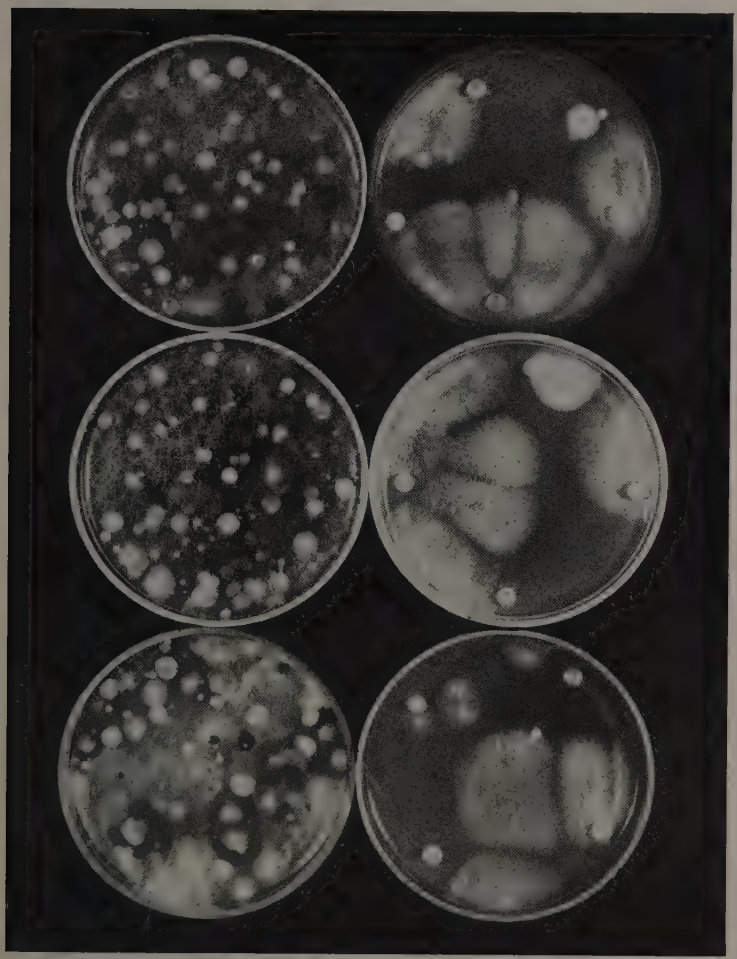
Microphotography as an Aid to the Identification of Trombiculine Larvae. (See page 298.)



New Species of *Paraschongastia* found on one bird in Central Queensland. Mite almost fully engorged. Mag. 100 ×. Scutum Mag. 610 ×. Note the obvious difference in the antero-lateral setae between this species and that depicted in Plate 5, also how clearly the oblique lighting used for these photographs shows up the detail of the shield including the ridges from which the sensillae arise in this genus.

**PLATE 7.**

Differential Isolation of *Chaetomium* spp. from Mixed Populations by Hypochlorite Solution. (See page 310.)



Plates exposed ½ hour on laboratory floor and incubated five days. Left—control plates incubated without treatment. Right—Plates exposed with controls, but treated with 4 glass beads bearing hypochlorite solution before incubation.

[*Photo. by T. Pickard.*

## PLATE 8.

Differential Isolation of *Chaetomium* spp. from Mixed Populations by Hypochlorite Solution. (See page 310.)

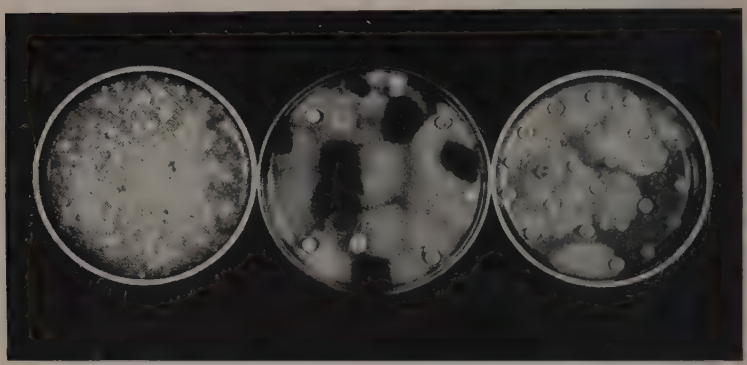


FIG. 1.—Plates exposed 1 hour on laboratory floor and incubated eight days. Left—control. Centre—treated with 4 beads bearing hypochlorite before incubation. Right—treated with 25 beads bearing hypochlorite before incubation.

[*Photo. by T. Pickard.*

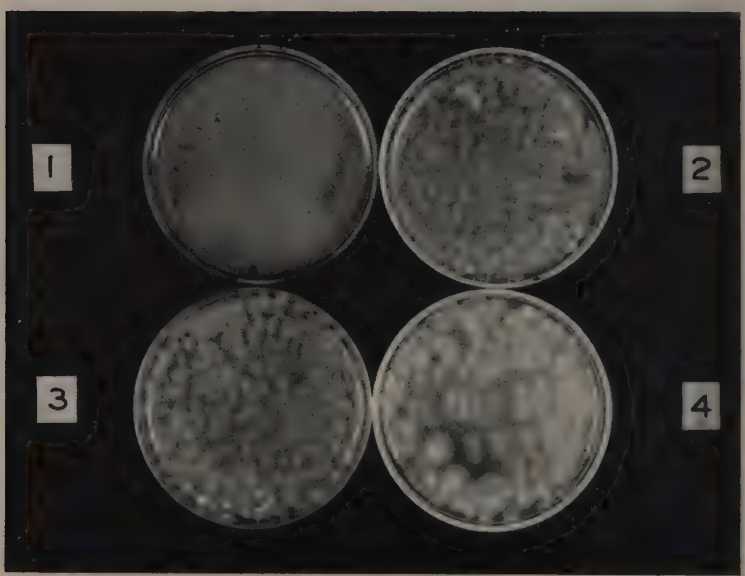



FIG. 2.—Plates treated with 1 cc. of hypochlorite solution bearing spores of *Chaetomium globosum*. Nine days after treatment. 1. Control—clean hypochlorite solution. 2. Spores in solution 5 minutes. 3. Spores in solution 10 minutes. 4. Spores in solution 15 minutes.

[*Photo. by T. Pickard.*

**PLATE 9.**

Mineral Deficiency in Plants on the Soils of the Ninety-mile Desert.
(See page 336.)

FIG. 1.—Laffer Sand, near Keith, South Australia. Natural vegetation on the experimental area. January, 1943.

FIG. 2.—The same area as shown in Fig. 1, in May, 1943, after cultivation procedure described in the text. Firm and level surface obtained by the cultipacking operation.

Mineral Deficiency in Plants on the Soils of the Ninety-mile Desert.
(See page 336.)


Showing the effect of superphosphate on the development of Algerian oats on Laffer sand on August 30, fourteen weeks after sowing.

Fig. 1 (above).—No fertilizer.

Fig. 2 (below).—Superphosphate 2 cwt. per acre.

## PLATE 11.

Mineral Deficiency in Plants on the Soils of the Ninety-mile Desert.
(See page 336.)

Fig. 1.—Showing the effect of superphosphate and zinc sulphate on Algerian oats on Laffer sand. Plants from 25 sq. links. Left of marker—no zinc.; right of marker—zinc sulphate 7 lb. per acre; and in each case, left: no superphosphate; centre: superphosphate 1 cwt. per acre; and right: superphosphate 2 cwt. per acre.

Note dark colour, particularly of lowest leaves, of plants without zinc.

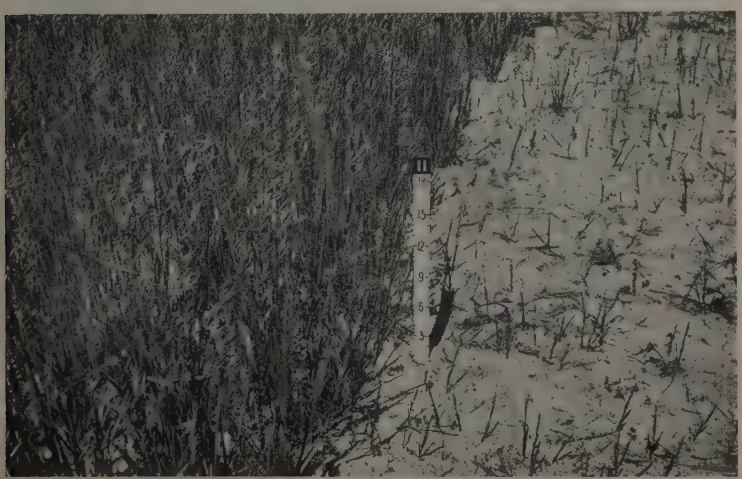


Fig. 2.—Showing the effect of superphosphate on Guyra oats on Laffer sand, 20 weeks after sowing. October 11, 1944. Left: superphosphate 2 cwt. per acre. Right: no fertilizer.

**PLATE 12.**

Mineral Deficiency in Plants on the Soils of the Ninety-mile Desert.
(See page 336.)


Showing the effect of superphosphate on the development of Ford wheat on Laffer sand, 20 weeks after sowing. October 11, 1944.

Fig. 1 (above).—No fertilizer.

Fig. 2 (below).—Superphosphate 2 cwt. per acre.

PLATE 13.

Mineral Deficiency in Plants on the Soils of the Ninety-mile Desert.
(See page 336.)


Showing the effect of zinc sulphate on Algerian oats sown on Laffer sand with 1 cwt. of superphosphate per acre. Plants from 25 sq. links.

Left: No zinc. The oldest leaves were dark brown; the upper leaves, stems, and panicle-branches were red or purple.

Right: Zinc sulphate 7 lb. per acre. Plants taller and much less discoloured.

The Etiology of Take-all Disease of Wheat. 1. (See page 318.)

Aerial view of the two-acre block in a take-all affected field at Canberra.

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